Secrets of the Heart

Cardiovascular Research

Medical Research Council of Canada
Conseil de recherches médicales du Canada

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David Spurgeon

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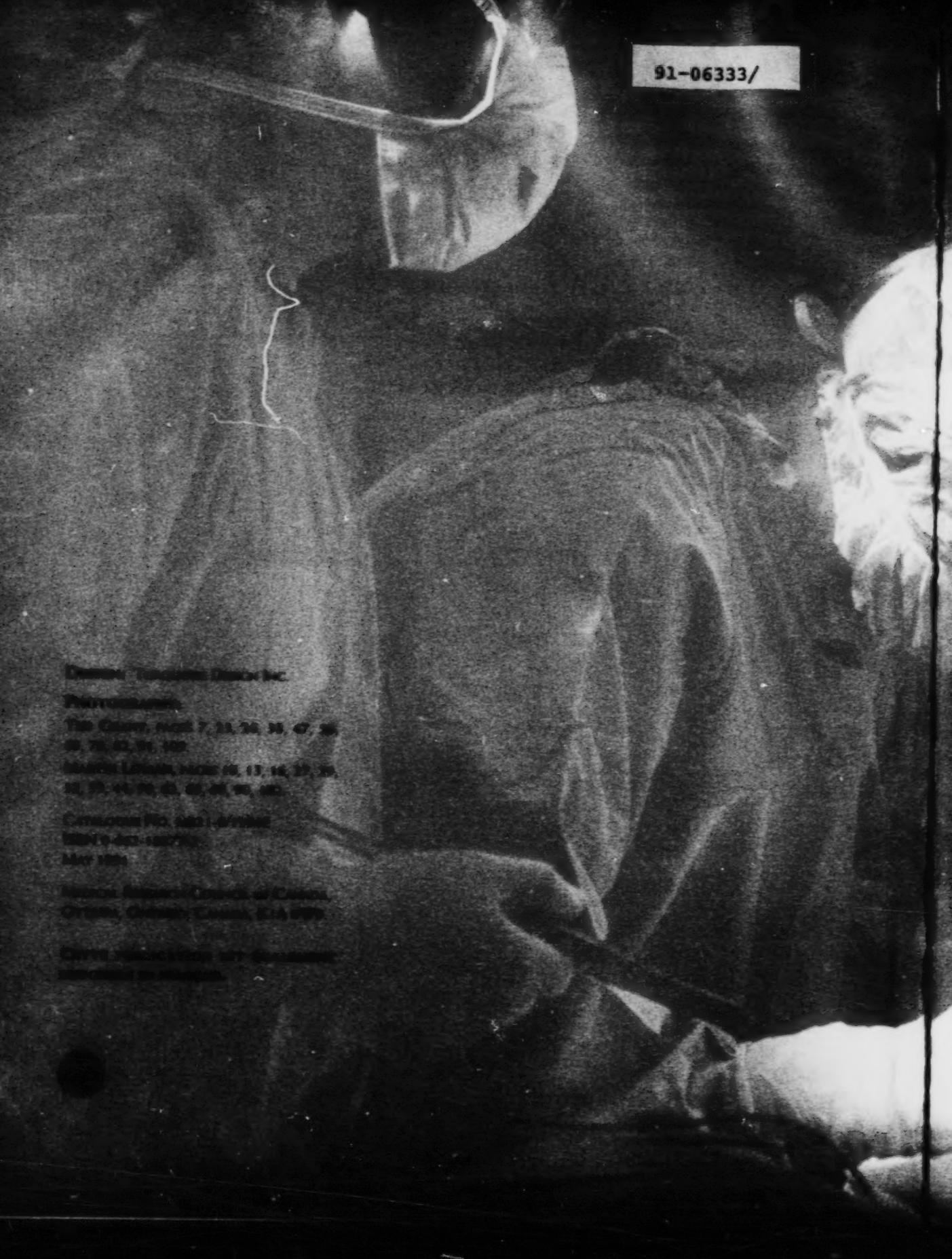

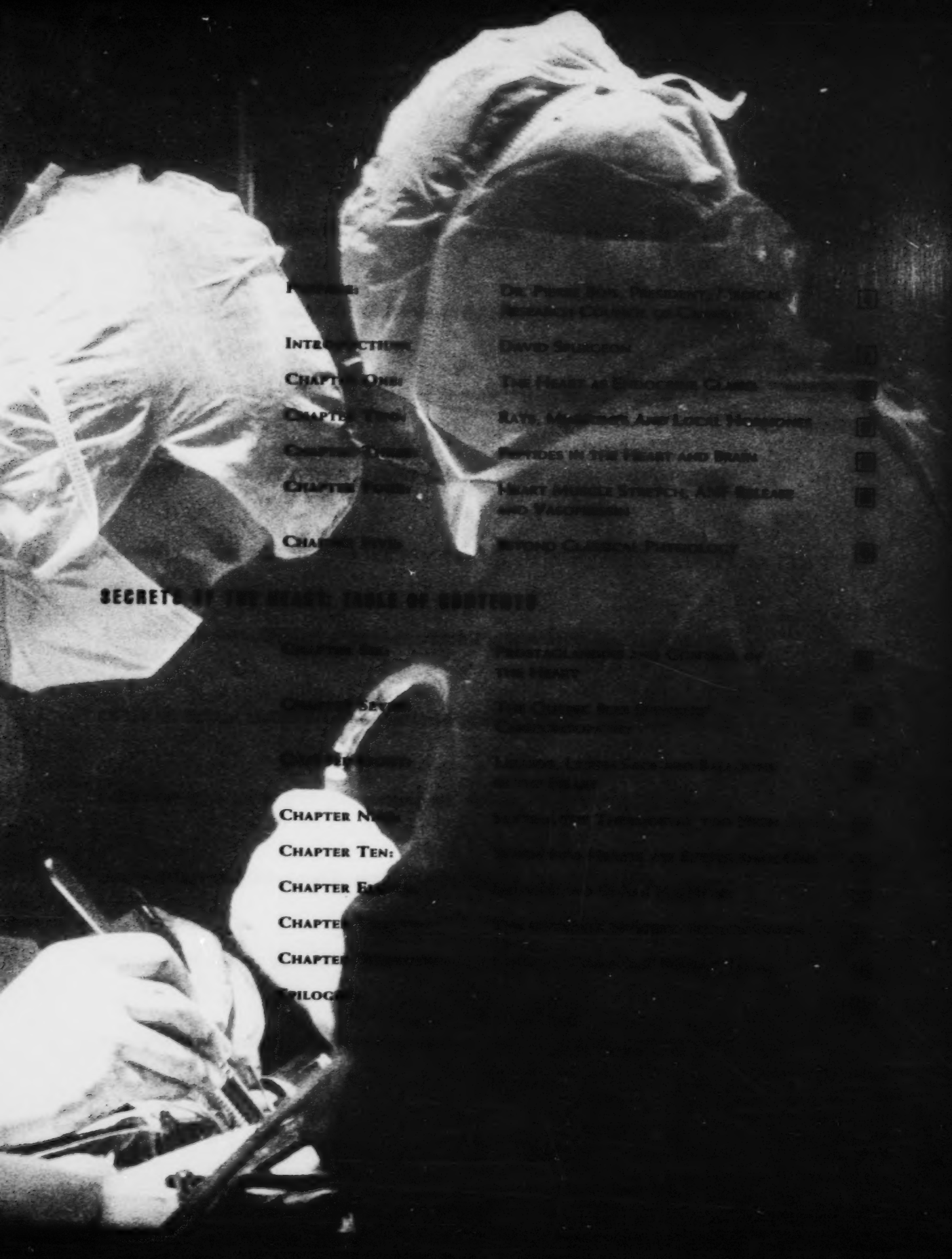

Introduction: David Shannon

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PREFACE: PIERRE BOIS, PRESIDENT, M.R.C.

Despite the great progress made in understanding and treatment of cardiovascular diseases in recent years, these diseases remain the major cause of death among Canadians. In 1988, the latest year for which statistics are available, they were responsible for more than 40% of all deaths in this country. This compares with 48.8% in 1970. It is for this reason that cardiovascular research is of special importance to the Medical Research Council of Canada (MRC), which with the Canadian Heart and Stroke Foundations supplies much of the financial support for such research. The projects described in this book represent a selection of cardiovascular research projects from across the country. While many others of equal merit and interest, such as pioneer work on cardiac assist devices ("artificial hearts"), might have been included, practical considerations precluded an

exhaustive treatment. Those presented here constitute part of the Canadian contribution to the field, some of which have already made a significant impact internationally. I wish to thank all those who have generously contributed their time for interviews. My hope is that this publication will help Canadians to understand the efforts being made to reduce the suffering caused by cardiovascular diseases, and also that it may serve to attract more researchers to this field – especially young people currently considering a career in research. In discussing the rewards and difficulties involved in such a career, many of those interviewed were exceptionally frank. Another way in which this book might be helpful is in helping Canadians decide whether they are providing enough support – and the right kind of support – for their medical research community. ***Pierre Bois, President, Medical Research Council of Canada.***

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In this year (1391), died likewise suddenly, the noble and gallant count de Foix. The day he died, he had all the forenoon been hunting a bear, and it was late in the evening when he was taken and cut up. The count with his companions rode a foot's pace towards the village of Rion, and dismounted at the inn. The count went to his chamber, which he found ready strewed with rushes and green leaves; the walls were hung with boughs newly cut for perfume and coolness, as the weather was marvellously hot, even for the month of August. He called for water to wash, and two squires advanced. The count rose from his seat, and stretched out his hands to wash; but no sooner had his fingers, which were handsome and long, touched the cold water, than he changed colour, from an oppression at his heart, and, his legs failing him, fell back on his seat, exclaiming, "I am a dead man: Lord God, have mercy on me!" He never spoke after this, though he did not immediately die, but suffered great pain... In less than half an hour he was dead.'

Six hundred years after the event described above, sudden death from heart disease is still a mystery. In the words of Dr. D. George Wyse, Head of the Cardiology Division of Foothills Hospital in Calgary, Alberta: "Somebody can come into my office this afternoon and I can see them and everything looks wonderful. I could do an electrocardiogram and even a treadmill test that look perfectly normal and they could walk out and drop dead. It happens. And we don't really understand why".

This in an age when surgeons have not only repaired ailing hearts but transplanted them from one human being to another; when the rate of deaths from heart disease and stroke has been made to decline by half over the past 30 years; and when a Canadian basic scientist with the appropriate name of de Bold has succeeded for the first time in showing that the heart is not just a pump, believed for centuries to be its only function, but is also an endocrine gland, producing hormones until then completely unknown to man.

The juxtaposition of the fantastic advances in knowledge of the heart and circulatory system made this century and our utter incomprehension of certain basic cardiophysiological processes, demonstrates one of the fundamental paradoxes of modern medicine. The pessimist may say that it shows the futility of man's endeavors. But the optimist – backed by the millions of patients who have benefited from medical progress – will declare, on the contrary, that the paradox proves the need to carry on ever more assiduously with the research effort.

Throughout history, the heart has been considered in a very real sense the fountainhead of life. In his famous *Textbook of Pathology* (1961 edition), the great Canadian physician William Boyd noted:

"Our language testifies to this fact, particularly the languages of the poets. We say that a man is broken-hearted, or wounded to the heart, or that he wears his heart on his sleeve, or that it is in his throat or in his boots. The heart beats 100,000 times a day, and yet it rests (during diastole) twice as long as it works. Our knowledge of the anatomy and physiology of the heart has advanced to an incredible degree. We can pass catheters into its cavities, record the electrical changes in its muscle, and estimate its enzymes. But we still do not understand how it is that one man may have extensive myocardial fibrosis without suffering unduly, while in another who dies apparently of sudden heart failure, no adequate explanation can be found at autopsy".

Until the middle of this century, the heart was also considered inviolable. In his autobiographical book *Cold Hearts: The Story of Hypothermia and the Pacemaker in Heart Surgery*, the pioneering Canadian heart surgeon Dr. W.G. (Bill) Bigelow

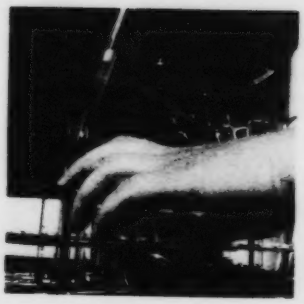
(see p. 11) recalls the pronouncement by the Viennese surgeon Theodor Billroth, one of the most influential teachers of the last half of the 19th Century: "Whoever attempts to operate on the heart will fail". One hundred years later, in 1949-50, says Bigelow, "surgeons were just losing their fear of the heart and very little was known about its anatomy and physiology. After all, why study an organ that is beyond human management?" During his work on hypothermia (artificial lowering of body temperature) which made surgery possible on the heart, he remembers a revered professor stopping him in the hospital corridor and asking: "Bill, do you really think you will ever be able to operate successfully upon the heart?"

Although it is now known that heart disease has a very long history (signs of arteriosclerosis have been found in Egyptian mummies), real understanding of the disease process is remarkably recent. According to the famed American cardiologist, Paul Dudley White, heart disease as such was not recognized until about 1500 A.D. Anginal pain (caused by narrowing of the arteries to the heart) was first recognized as such only in the second half of the 18th Century, and myocardial infarction (death of the heart muscle caused by blockage of its blood supply) early in the 20th.[2] Even as late as the early part of this century the term "heart attack" was unknown: victims of myocardial infarction were commonly said to be suffering from "apoplexy" or "acute indigestion".

J.O. Leibowitz notes in *The History of Coronary Heart Disease* some "facts and concepts which led ultimately to the recognition of anginal and myocardial disease":

"In ancient Egypt, apart from archaeological evidence, the occasional notion of precordial (abdominal) pain with 'death that threatens him', many Biblical references to a sore heart, the vast majority of them poetical, but some also of medical implication, and Talmudical references to heart pain as meaning disease, the Hippocratic concept of obstruction, which includes vascular obstruction, and the Hippocratic mention of sudden death probably due to an anginal episode, then we come to Galen with his clear anatomical description of the coronary vessels along with their nutrient function, the Graeco-Roman syndrome of 'morbus cardiacus' seems to include the later notion of shock... And to the genius of Leonardo da Vinci we owe the pictorial representation of narrow and tortuous peripheral vessels in the aged and the harmful influence of this condition on the tissues..."

By the 18th Century, says Leibowitz, interest in cardiology had increased, and textbooks had appeared on the heart and its diseases. But although both angina pectoris and myocardial infarction were known as separate entities, it was not widely accepted that a blood clot combined with narrowing of the



coronary arteries could cause death of the heart muscle. One of the pioneers in publicizing this concept, the American Dr. James B. Herrick, recalled long after he presented what is now considered his classic paper: "When it was presented in 1912, it was discussed by only one person, there was no repercussion for six years. It had fallen like a dud". Leibowitz, after quoting Herrick, adds: "Even later the repercussion was limited to a small group of researchers while the general acceptance in medical practice came only in the 1930s".

It also took time for the medical profession to recognize that the results of coronary thrombosis were not always fatal. In 1933, Dr. D. Evan Bedford wrote: "The knowledge that coronary thrombosis is often compatible with life and even health, and that it can be recognized during life with certainty in many cases, has only come to us during the last twenty years".

White argues in *My Life in Medicine* that coronary heart disease itself could not have been all that common in the early part of the 1920s and earlier, though this is disputed by some. White also recalls that cardiology (the scientific study of the heart) was still considered by most medical leaders in the 20s "as trivial preoccupation and not likely to amount to anything but rather a waste of time".

Cardiology is relatively new to Canada. In a letter to Dr. Harold Segall in 1972, Dr. David Baltzan wrote: "The specialty did not exist in Western Canada 50 years ago".

The greatest advances in the understanding and treatment of cardiovascular disease began to be achieved with the organization and funding of biomedical research as we know it today, after the second world war. The discovery of antibiotics played a part: with the availability of these remarkable drugs, beginning with penicillin during the war, rheumatic heart disease became preventable. Caused by a streptococcal infection, the fever that led to rheumatic heart disease could be quickly cured. Says Boyd of the related condition, bacterial endocarditis:

It used to be a malady in which the heart was beating muffled marches to the grave...Now, thanks to antibiotics, it has become a curable disease... This, as Maxwell Finland remarks, is one of the great triumphs of antibiotic and chemotherapy.

Canadians have figured prominently in the advances made in research and treatment of heart disease since the second world war, especially in heart surgery. Dr. Maude E. Abbott (1869-1940), termed by Segall "the most famous Canadian cardiologist", was credited by White with being "the

most important of the pioneers in establishing congenital heart disease as a living part of clinical medicine".

The use of heparin – an anticoagulant substance that occurs naturally in the body – in the treatment of thrombosis, with artificial kidneys and in the extracorporeal circulation necessary for organ transplants and heart surgery, was a Canadian development. The use of hypothermia in heart surgery and the development of the artificial pacemaker to control heart rhythm were pioneering efforts of Bill Bigelow[3] in Toronto. Dr. Gordon Murray of Toronto was the first to replace a section of a coronary artery surgically as early as 1940, and later, in the 1950s, became the first to attach another artery to a coronary artery. The first mammary artery implants to improve blood supply to the heart muscle, and thus reduce anginal pains, were done by Dr. Arthur Vineberg of Montreal – a development that led to coronary by-pass surgery. The first use of prostaglandins to help correct a congenital condition in babies was by Drs. Peter Olley and Flavio Coceani in Toronto (see Chapter 6). And a major contribution to congenital heart surgery was the procedure developed by Dr. Bill Mustard of Toronto for correction of blood-flow defects in the so-called "blue babies".

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The changing picture of heart disease during the past 30 years became apparent to me during the preparation of this book. In 1960, when I conducted interviews with researchers across the country for *The Globe and Mail* in collaboration with the Heart Foundations of Canada, the four most common forms of heart disease in Canada were listed as congenital conditions, which are present at birth, rheumatic heart disease, hypertension (high blood pressure) and those due to atherosclerosis (hardening of the arteries), which restricts blood flow and may lead to heart attack. Three of those conditions – atherosclerosis, hypertension and rheumatic fever – were said to cause more than 90% of all heart disease.

This year (1990), those interviewed agreed that rheumatic heart disease is no longer a major problem in Canada. As Dr. Michael Sole, Director of the Division of Cardiology and the Centre for Cardiovascular Research at The Toronto Hospital, put it: "We don't see acute rheumatic fever in our patients: I haven't seen one for years...". Added Olley: "I think we've solved that one (rheumatic heart disease). I think we can say that we fully understand this and it's virtually been eliminated from Canada".

The same cannot be said for the other two conditions, however – hypertension and atherosclerotic heart disease. They still head the list in incidence of heart problems in Canada, and their basic causes are still unknown. "There've

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been some very significant advances", said Olley, "but as basic problems we really do not know what causes high blood pressure in most patients". Added Wyse: "We think we have some idea how to prevent atherosclerosis but we're not entirely sure yet, and we're not entirely sure what the impact of that will be. Whether we'll ever reduce those things (hypertension and atherosclerosis) to zero or close to zero, I don't know".

Nevertheless, the statistics of heart disease are encouraging. Over the past 30 years, fewer Canadians have been dying from heart disease and stroke: deaths due to heart disease in this period have declined by 53% in women and 34% in men, while for stroke the decrease has been 50% in both men and women.

What this shows is that, despite ignorance of basic causes and uncertainties about some treatments, the results of research have been taking us in the right direction. Not only has rheumatic heart disease been all but banished from the young, but for those who contracted it years ago the therapies now available are much better than they were. And though the causes of most cases of hypertension may remain unknown, thanks to research and new drugs it is much better controlled today. "It's very rare that we see a patient with malignant hypertension or somebody with a hypertensive crisis coming to the emergency department", said Sole. "We just don't see that any more".

One of the biggest conundrums remaining is the cause of atherosclerosis, and the effects of lifestyle on its development. Knowledge of the facts is far less certain than is implied by television commercials for oat bran cereals and low-fat foods. Thus while it is known that the recent reduction in deaths due to heart attacks and stroke is real, and that there is a relationship between heart disease, diet and other aspects of lifestyle, these cannot be equated for any one individual. But, says Wyse:

"You can do it in a population. You can say if everybody in Alberta smoked less, lost weight, were fitter, had better control of blood pressure and diabetes, there'd be fewer people dying of heart attacks in this province. But if I go to you, the person, and say if you did all those things you're less likely to die of a heart attack, I'm not sure... There are some things we don't understand. We don't understand, for example, those narrowings that develop in the arteries; they come silently initially. We know from autopsy studies done during the Korean War that they begin in 18- and 19-year-olds. But if you've got a 75% narrowing in an artery, it's probably been there for weeks, months or years. What makes it on Wednesday, July 4 at 12:30 suddenly block off? Why didn't that happen yesterday or two weeks ago? We don't understand that. And we

don't understand how to find that person. If he's never had a chest pain, if he's never had anything to suggest that there's anything wrong with his heart, how do you go about picking those people out of the general population?"

Olley agreed: "It's certainly true that there's a lot we don't know, but I think it's also true that we know enough to say that in general terms the lower your cholesterol the better. So we should be trying to influence people through their diet, through physical activity, into lifestyles and diet that will reduce the population's cholesterol. The problem is when you come to deal with individuals. We all see patients who have low cholesterol but they have a heart attack, so it's obviously not the whole story. But dealing with the population as a whole we know that those countries where the average cholesterol is lower than in Canada have less heart attacks and stroke".

This much is certain: heart disease is still the leading cause of death in Canada. Statistics Canada figures show cardiovascular diseases caused 41% of all deaths in this country in 1988. Cancer was not even close, causing only 27%, while respiratory diseases were third with 8%, accidents fourth (7%). All other causes made up only 17% of the total.

As well as deaths, heart diseases cause untold personal suffering. And they are prodigiously expensive. Hospital admissions account annually for millions of days of hospital care. Estimates of their cost in Canada may be as high as $26 billion a year, according to the Heart and Stroke Foundation of Ontario.

Recently, some of those involved in combatting heart disease in Canada have begun to fear that Canadians may be forgetting the continuing need for research.

"I spend a lot of time with the Heart Foundation and we're very concerned about the national image of cardiovascular research", said Olley. "We seem to be forgetting that, of Canadians who die, one out of every two is killed by heart disease or a stroke – many more than cancer, certainly many more than AIDS. While we've made tremendous strides there's still a long way to go, and I think somehow the federal agencies and the public perhaps need to be reminded that cardiovascular disease is still a major public health problem".

The purpose of this book is to serve as such a reminder – and to show through the examples that follow what some of Canada's leading researchers are currently doing to try to reduce the suffering and deaths caused by heart disease.

The first three chapters deal with a Canadian discovery: that the heart produces a hormone called atrial natriuretic factor (ANF). This totally unexpected finding has attracted worldwide attention and stimulated an enormous amount

of new research, some of which is expected to greatly increase our understanding of both the normal workings of the cardiovascular system and of a multitude of its malfunctions. A number of other chapters (for example, Four and Five) deal with research that arose out of the ANF discovery, or with associated phenomena.

Some of the work described here is extremely basic in nature (see, for example, Chapters Eight and Ten). In other cases (e.g., Chapter Seven) it is highly practical. Much (see Chapters Six, Nine, Eleven and Twelve) is a combination of both.

Some of the work – for example, that of Dr. Réginald Nadeau in Chapter 12 – shows how laboratory concepts drawn from basic research can be applied directly in treatment of patients: his studies have not only illuminated the underlying mechanisms of arrhythmias but have helped to identify where in the heart they arise. "We've tried to combine the experimental and clinical aspects as soon as possible in mapping of the heart and arrhythmia surgery", Dr. Nadeau said.

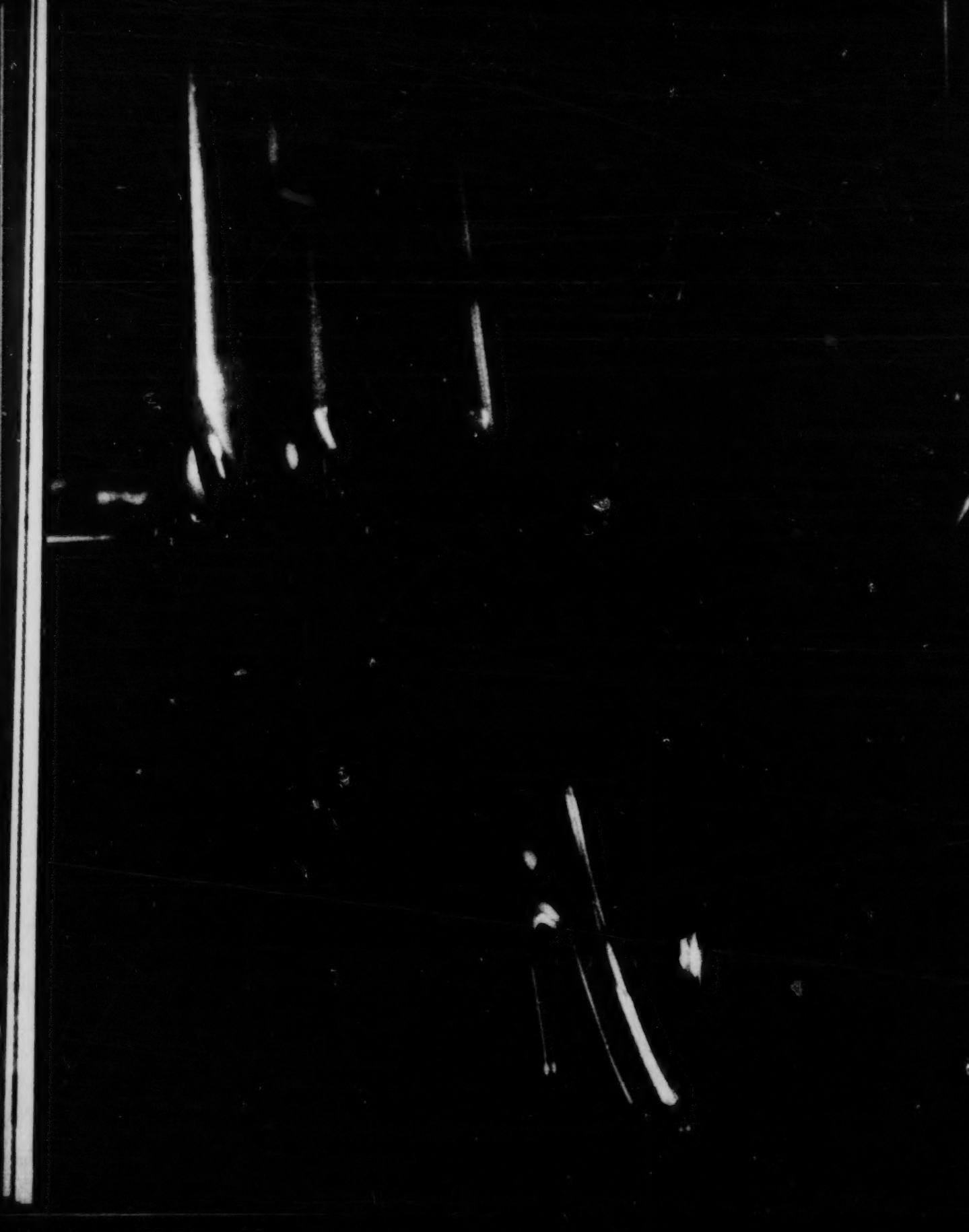
Dr. Jean L. Rouleau's work at the University of Sherbrooke provides another such example: his combination of laboratory studies with animals and clinical ones with patients who have suffered heart attacks is both clarifying how apparently successful drugs work, and helping to treat patients. "Some of the drugs are already used with patients", said Rouleau, "but we don't understand why they work – like beta blockers. Beta blockers prolong survival, especially for people with big infarctions, but we don't know why. It's difficult to get a rational combination of medications if you don't really know why a drug works".

The final chapter tells how practitioners can determine which are likely to be the best choices for an individual patient among the many treatment options that all this medical research produces. The most valuable tool they have yet devised for this purpose is called the randomized clinical trial. As Dr. Martial G. Bourassa of the Institut de Cardiologie de Montréal puts it: "Nothing is perfect. But a well-performed clinical trial eliminates bias and makes the findings credible".

[1] Froissart, Sir John. *Chronicles* (ca 1400).
In: Leibowitz, J.O. *The History of Coronary Heart Disease*, London,
Wellcome Institute of the History of Medicine, 1970 (abridged).

[2] Dr. Harold N. Segall of Montreal in his *Pioneers of Cardiology in Canada 1820-1970*, quotes Sir William Osler on the rarity of angina pectoris in the late 1800s: "During the ten years I lived in Montreal, I did not see a case of the disease either in private practice or at the Montreal General Hospital".

[3] The artificial cardiac pacemaker delivers regular electrical impulses to electrodes implanted in the heart to stimulate heart muscle contraction in patients whose physiological pacemaker fails to function properly. In *Cold Hearts: The Story of Hypothermia and the Pacemaker in Heart Surgery*, Bigelow describes how Dr. John Callaghan, Jack Hopps, an electrical engineer from the National Research Council of Canada and he designed and tested " the first pacemaker for continuous human use with a controlled physiological electrical stimulus that caused no injury to the heart muscle". The pacemaker was developed in the course of Bigelow's hypothermia experiments to overcome the problem of cardiac arrest when the body temperature of the experimental animal was reduced below about 24 degrees C. Their report on the project was one of the scientific highlights of the Annual Surgical Congress of the American College of Surgeons in Boston in October, 1950. Writing in *The Lancet*, March 19, 1988, surgeon Hans Lagergren of the Karolinska Institute's Huddinge University Hospital, noted that since a simplified insertion method had been invented there in 1962, "more than 200,000 pacemakers are implanted (worldwide) every year, and over 3 million transvenous implants have been inserted".



THE HEART AS ENDOCRINE GLAND

For more than three centuries after William Harvey described the human body's circulatory system in 1628, the heart was considered to be simply a pump. However vital its function, it was believed to do nothing more than circulate the blood. In 1980 an event occurred in Canada that changed this concept of the heart forever: the discovery by an Argentinian-born researcher, Dr. Adolfo J. de Bold, working at Queen's University, that the heart produced a hormone called atrial natriuretic factor (ANF). Rather than being simply a pump, the researcher showed, the heart was also an endocrine gland. The concept was revolutionary. Said Dr. John R. Ledsome, of the Department of Physiology at the University of British Columbia: "I suppose ANF was more of a surprise than the discovery of insulin... We had been looking for 20 years for a so-called natriuretic hormone: I personally spent a lot of time taking out every organ in the body to try to identify the source of this natriuretic substance. The one thing we didn't take out was the heart. The whole concept was foreign, that the heart should produce a hormone". De Bold now is the director of the University of Ottawa Heart

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Institute Research Centre at the Ottawa Civic Hospital. After working virtually alone for some years to determine the function of granules located in the atria of the heart, he found that they secreted a substance that caused the kidneys to excrete increased amounts of sodium in the urine (an activity known as natriuresis – hence the hormone's name). ANF was a powerful diuretic which could lower blood pressure, among other properties.

As Ledsome suggests, a substance with some such properties had long been suspected.

"The first observation about the differences in the atrial muscle (the observation of the granules that produce ANF) was made in 1955 but no one knew what it meant", said de Bold recently. "And the reason they didn't, I suppose, was because no one was willing to spend the time finding out. I had the advantage that I had had multidisciplinary training and so could do many things without help, so to speak".

De Bold's discovery touched off what Dr. Geoffrey Flynn, of Queen's University's Biochemistry Department calls "an entirely new field: the heart as an endocrine organ", which is different from anything else going on in cardiovascular research.

De Bold's first paper on the discovery was published in 1981. Isolation, purification and sequencing of the amino acids that make up the peptides that comprise it were reported in 1983 by de Bold and Flynn (see Chapter 3). Since then, de Bold reported at a 1990 Ottawa symposium entitled "A Decade of ANF Research", some 3,700 papers dealing with ANF had been published.

Besides its potential in revealing new knowledge about the heart and the circulatory system, the ANF discovery prompted an immediate flurry of activity among pharmaceutical manufacturers, who saw a multi-billion-dollar market for a drug that could mimic ANF's blood-pressure-lowering effects. Those closely involved with ANF thought it might become, as one of them put it, "the anti-hypertensive drug of the century".

This initial enthusiasm has been tempered, because it was soon realized that, being a peptide, ANF could not be taken by mouth: just as with insulin, the digestive system would destroy it. Pharmaceutical firms then turned to different approaches: one was to try to make an analog of ANF that would resist the digestive process. The other was to develop new compounds that would inhibit the breakdown of ANF in the body so as to preserve its blood-pressure-lowering effects. These efforts are continuing. De Bold's own institute is testing some of the compounds in clinical trials.

But even more important have been the new avenues of research the ANF discovery has opened up.

"It's incredible the amount of work that has been generated", said de Bold recently. "Not only did we have a new hormone but we had a new concept altogether: first that the heart was an endocrine organ; second, atrial natriuretic factor was a hormone with a very wide spectrum of action. It interacted with just about everything in the body that had anything to do with the control of blood volume and blood pressure. Also ANF produced the unexpected finding that it acted through a unique transduction mechanism. Then it turned out that ANF is present not only in the heart but also in many tissues including the brain, in which it appears to control water and salt appetite, so it also has behavioral consequences. These peptides are present in areas in the brain that have nothing to do, as far as we know, with water and electrolyte balance. So this is a sort of peptide of universal use".

The complexities of the ANF story multiplied with the report by a Japanese investigator, Hisayuki Matsuo, that he had discovered two new natriuretic peptides, which he called BNP (brain natriuretic peptide) and CNP (C-type natriuretic peptide). He refers to all three, which are distinct types, as members of the natriuretic peptide family.

The question is, what exactly do all these newly-discovered hormones do? What is their mechanism of action, and where do they fit in with the body's other regulatory systems?

The release of ANF seems to be produced by the stretching of the heart's atrial muscles, though no one knows exactly how. This in itself seems paradoxical, because atrial stretch is considered to be a rather crude mechanism for production of such an apparently important hormone.

The more researchers delved into the mysteries of ANF, the more paradoxical the picture became.

"We still know remarkably little about exactly what the actions of ANF are on the circulation and the kidneys", said Dr. Andrew Rankin of Memorial University of Newfoundland. "How is it causing the diuresis and natriuresis? We're still not entirely clear about that after 10 years. We're still not entirely clear about how it might be lowering blood pressure. It's still very confusing".

Ledsome asks: "What is ANF doing in the brain? The receptors in the brain (to which ANF binds) are localized in those areas that we have known for 20 years are involved in control of water and salt excretion. So the binding sites in the brain are in all the right places but we still have no idea if in fact the ANF that is circulating in the blood, or the brain ANF, is affecting

those binding sites in the brain, or what the brain is doing to control the vascular system and the kidney in response to those sorts of signals.

"And it may be that ANF's direct actions on the kidney or the blood vessels are really irrelevant. Maybe it is acting through the central nervous system. It's in the eye, it's in the testes – what's it doing in those other sites? There's a lot of it in the lung – ANF is actually produced in the lung in quite large amounts. Is it affecting water movement in and out of the air spaces, or the capillaries, because water movement in the lung is extremely critical?"

Thus the original focus in ANF research on the hormone as released by the heart and circulating through the body like a chemical messenger, as do other hormones, has now broadened. Or narrowed, depending upon how you look at it – to the question of the receptors, for example, the sites to which the hormone molecules bind. The Ottawa symposium was full of discussion on ANF receptors. Said Olley:

"People are now describing receptors for everything under the sun, and it causes you to step back for a moment to say, 'Did God really design things that way? Is it as complicated as all that?' Maybe it is, but I think it's very important before you embark on something that's going to cost thousands of dollars and two or three years' work, to be sure you are following something real. There are hundreds of people working on ANF now, but its exact role is still quite a mystery. One would like to see somewhere at the end a practical application".

" You might say this is now the age of teamwork, and that there's too much knowledge to digest for a single person to be out there doing what people did in the 19th Century and earlier. "

His view is echoed by Sole, who says that we know what ANF does in terms of the kidneys and as a vasodilator (enlarging the blood vessels). "But describing these actions and then demonstrating that this is unequivocally important in day-to-day physiological regulation in humans are two different things. And it's that latter one they're having a little difficulty with".

ANF also has larger implications, Sole says: it is produced intracellularly and it may have an important role as a factor in growth and development. It is found in cells of primitive organisms. "So one area of interest in research is, what's the role of ANF as a growth or developmental regulator inside the cell?"

Sole points out that the heart is not just muscle. Myocytes, or heart muscle cells, constitute only 25% of the cells in the heart: 75% are other cell types. "We're getting very interested in the cardiac matrix – the skeletal framework of the heart – the collagen matrix in which the muscle cells lie. That matrix is not a random one, it's very organized. You could also call it a skeletal structure that gives the heart its form and plays a role in its function.

"One of the major changes that has occurred over the last 20 years has been in our shift of focus from the physical properties of the heart as a pump, to molecular processes that are important for the growth and development of the heart, and the relationship of these processes to those in other tissues.

"Another earlier change was the realization that the neural control of the heart by reflex mechanisms and the brain, in essence its wiring, is also very important. And so you have to really look at the circulation as an integrated neuro-regulatory apparatus of which the heart is really one component.

"In the past the heart was treated as an island. I think now we recognize it as a component – in fact it's a peninsula, attached to and dependent on the rest of the circulatory system. We used to ignore the nerves and the vessels and its attachments".

Sole, who started his professional life as a physical chemist, compares the growing understanding of the heart with that of particle physics. "We went through a phase where we described more and more fundamental particles. And only as we began to develop an understanding did we suddenly begin to see them as parts of larger families, and order once again appeared where initially there appeared to be ever-increasing disorder. I think the same thing is true when you begin to dissect processes on a molecular level".

As a basic scientist, de Bold holds similar views.

"In terms of understanding the major causes of heart disease", he said, "we are not much further ahead than we are with many other diseases. In order to really go after these problems, ultimately you have to solve them at the basic science level. And cardiovascular diseases – as do cancer and AIDS – have molecular bases. It's not the same as going after a pathogen that causes an infection, which you can actually cure. In cardiovascular disease, as in cancer, what makes things go wrong is still something we have to unravel at the molecular level.

"What is interesting about the basic science at the molecular biological level is that the knowledge that is uncovered for any given process applies more or less across the board to all diseases and all physiological processes: if you are looking at gene control it is likely that what you discover is done in cancer will apply also to cardiovascular disease or to, say, a metabolic disease. So when you are doing basic science you are really advancing the field for the whole of biology".

What effects have the most recent advances in molecular biology had on cardiovascular disease?

"We know now that anything that produces an overload in the heart will lead to a series of molecular events. We are far from understanding this, but we can describe it in terms of what genes are activated, what proteins are synthesized ...so we are gaining a very detailed understanding. Heart hypertrophy (enlargement of the heart), for example, has taught us that not all hypertrophies are the same at the molecular level. We see that different stimuli produce a different set of proteins in the heart.

"What does that mean? It suggests that you cannot treat everyone in the same way, just as you cannot say that all cancer is the same. And that's a very important new concept to understand. Therapies will evolve, I am sure, tailored to these types of reaction".

Relating his ideas to his heart hormone discovery, de Bold says: "We went into the heart atrium muscle because we noted under the microscope that there was something different associated with the atrium muscle cell.The most obvious difference was that the atrium muscle cell had these storage granules that are found normally only in endocrine cells. That's all we knew. We had no idea whether or not studying this would lead to any new physiological phenomenon or any therapeutic approach or any industrial spinoff.

"The optimist would have said there must be a hormone to be discovered, while the pessimist would say you have no basis to expect that, therefore you shouldn't waste your time looking for it. We were optimists and we pursued that from 1968-1980, using a number of different approaches. I was at Queen's University, and I was blessed by circumstances – or by God or however you want to put it – with the fact that I was never under undue pressure to produce either an industrial spinoff or scientific publications. That gave me time to look into it with the type of dedication it required. Yet the discovery and use of ANF is a classical example of going from the basic science to the applied".

De Bold's current plans are to try to determine how muscle stretch – a mechanical stimulus – controls the synthesis of ANF, and how that is related to muscle hypertrophies (enlargements), such as heart hypertrophy.

De Bold's basic science approach was one factor that first set him on the trail of ANF. His graduate advisor's passion was another. After studying biochemistry in his native Argentina and moving in 1968 with his wife, Mercedes, to Canada, where he did postgraduate studies in pathology at Queen's University, de Bold, through the influence of Sergio Bencosme, became interested in the research that led to his discovery. Bencosme, a pathologist from the Dominican Republic, had long been intrigued by the clusters of microscopic granules found

in the cells of the upper chambers of the heart, the significance of which was unknown. And he suggested de Bold might want to look into the matter.

After finishing his doctoral thesis in 1973, de Bold accepted a research position at Hotel Dieu Hospital, which is affiliated with Queen's. He had no teaching responsibilities, so he was free to follow his new interest in the atrial granules. This he did with intense concentration and single-mindedness.

As Olley has indicated, de Bold (whose wife collaborated with him in his research) was not the only researcher looking for a natriuretic factor. Scientists at Yale University, the University of Paris, the Institut de recherches cliniques de Montréal (IRCM) and elsewhere were also on the trail of such a substance. It was a search that many thought skirted the edge of scientific respectability. "The concept of the endocrine heart you discussed only with your closest friends", de Bold told the Ottawa symposium.

Although he began by working on his own, others were later involved, including Flynn and Ledsome, Peter Davies, John Roder, Harald Sonnenberg. His chief rival in the ANF quest was Dr. Marc Cantin of the IRCM, whose sudden death – from a coronary thrombosis – came just prior to the Ottawa symposium, at which he was scheduled to give a research paper. Cantin, who headed the institute's multidisciplinary hypertension group, had been interested in the atrial granules since 1974. In 1988, Cantin received the Distinguished Achievement Award of the American Heart Association for his work in the field.

" in terms of understanding the major causes of heart disease, we are not much further ahead than we are with many other diseases. In order to really go after these problems, ultimately you have to solve them at the basic science level..."

But to de Bold goes the credit for having done what Flynn calls "the benchmark experiment", which Flynn says "showed brilliant insight": he pulverized some rat atria and extracted their contents in acid, which Sonnenberg, then at the University of Toronto, injected into the bloodstream of a rat to record the effect on the kidneys. What happened was that the kidneys excreted vast amounts of urine containing sodium ions, resulting in a drop in the rat's blood pressure.

"It was", de Bold said, "like turning on a water tap".

The crucial experiment that led to this result was in trying to extract the ANF from atrial tissue.

"Most polypeptide hormones are extractable in acetic acid", said Flynn, "and de Bold noticed that when he was doing his histochemistry staining of the granules and fixing his sections of heart tissue in acetic acid, he lost sight of the granule. And he said to himself: 'It's dissolved!' It was doing the acid extraction that gave the clue to develop the first chemical extraction method".

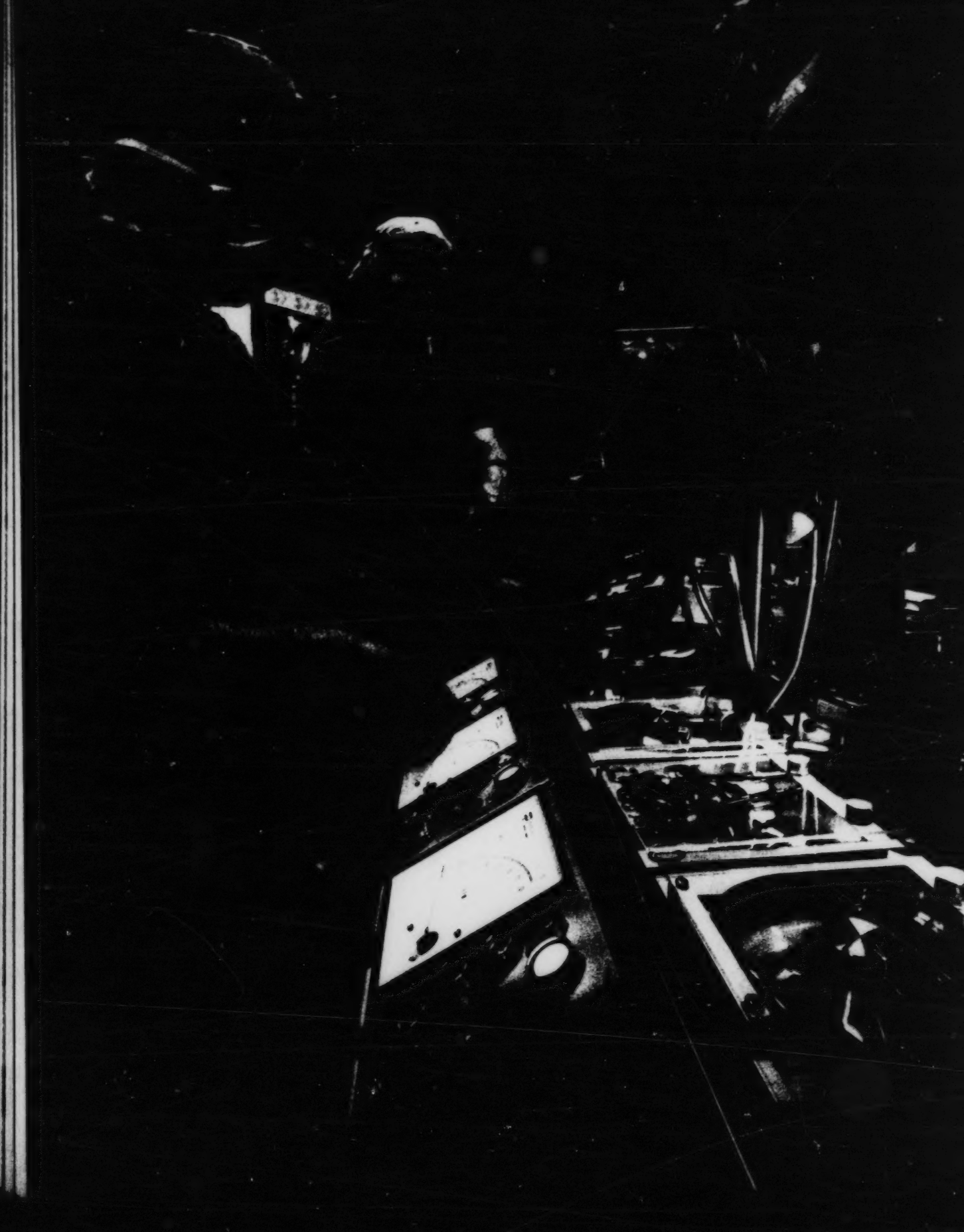
De Bold's approach to research was fundamentally as someone who worked alone, a method thought by many to be out-of-date but still valued by many others. Although he now heads a laboratory, he obviously feels somewhat ambivalent about the "big science" approach.

"You might say this is now the age of teamwork, and that there's too much knowledge to digest for a single person to be out there doing what people did in the 19th Century and earlier. And that may be true. I'm not sure, though, what will be accomplished by establishing bureaucracies... The bureaucracy is something useful that provides stability among other things. But it cannot provide the leadership needed".

De Bold "grew up in a society in which intellectual worthiness was worthwhile pursuing". His family valued such a pursuit, as well. And he remembers being fascinated by a picture of Louis Pasteur in his older sister's biology textbook.

"I always was interested in research", he said. "During high school I had my own lab in my home, where I did crystallization experiments. I had the chance to go into medicine or biochemistry and I picked biochemistry because I felt that was the way to go to solve problems – at the basic science level – even though I wasn't aware as I am today of the difference between basic and clinical science. So I guess I am wired for it!"

CHAPTER 1



"We had large amounts of synthetic ANF, which we could try out in all kinds of ways, and we established very fast all the peripheral effects of this peptide."

When Adolfo de Bold made his startling discovery in 1980, no one was more interested than Dr. Marc Cantin* of l'Institut de recherches cliniques de Montréal. Since the 1970s, Cantin had himself been hot on the trail of a natriuretic compound, though at that time, of course, the name ANF had yet to be coined. So when de Bold's discovery was made that ANF could produce diuresis (an increased flow of urine),

RATS, MINIPUMPS AND LOCAL HORMONES

Cantin and his group of hypertension researchers at IRCM were ready. In parallel with the Queen's University group, they succeeded in isolating and sequencing the peptide, and developed an arrangement with a pharmaceutical firm to obtain quantities of the synthesized hormone. Said Cantin: "We had an advance on everybody because we had large amounts of synthetic ANF, which we could try out in all kinds of ways, and we established very fast all the peripheral effects of this peptide". Cantin noted that ANF acts on all the targets known to increase blood pressure. "It's the first time that we have found one substance in the human body that seems to be so anti-hypertensive", he said. "All the other hormones that were known

were always pro-hypertensive: the renin-angiotensin system, aldosterone, vasopressin. Most of the peptides known produce increases in blood pressure, whereas this peptide seems to be there to dampen all these effects and to protect the body against hypertension".

Experiments have shown that repeated, relatively small doses of ANF will lower blood pressure in rats, monkeys and man, Cantin said. But the hormone cannot be given by mouth and injections would have to be continuous to be effective, which would not be practical, so the major pharmaceutical companies have been working on alternatives. The most promising is an enzyme inhibitor that would inhibit the degradation of the hormone that normally occurs in the blood. Unlike ANF, the enzyme inhibitor can be taken by mouth, and in trials it has successfully lowered blood pressure among people who have what is called essential hypertension (the term indicates simply that there is no known cause for the hypertension, and this category includes about 95% of all hypertensives).

Cantin said his group has found that ANF is produced by all the cells of the heart, not just the atria. "We have found that it inhibits aldosterone secretion, renin secretion, vasopressin secretion. It also dilates the blood vessels. The most interesting finding is that, when it is given over a long period to small animals, its levels in the blood do not increase. So we don't know its mechanism of action".

To study the ANF mechanism in the laboratory, Cantin said: "We give it by minipumps: these are pumps that are implanted in the rat's neck and deliver the peptide at a given rate in the circulation. We give it for several days, and after 24 hours the blood pressure of hypertensive animals goes down, and it stays down all the time we give the peptide.

"But if you measure the blood plasma levels, the degradation is so fast that there is no increase. So there must be some local effect, which we are trying to find now, which prevents the rise in blood pressure even though the plasma levels are not increased.

"We have found the same in monkeys, and a group in the Netherlands has found the same in humans, too. The dosage they give slightly raises the plasma level, but they found in six hypertensive patients the blood pressure returned to normal during the whole time ANF was infused".

Aside from ANF, Cantin spoke of another interesting approach to solving the problem of hypertension: an angiotensin-2 inhibitor devised by a U.S. pharmaceutical company. This is not a peptide and can be given by mouth. It blocks the receptors for angiotensin-2. In rat models of essential hypertension,

this compound brings blood pressure to normal, and the company is planning clinical trials in which IRCM will participate.

Renin is an enzyme produced by the kidney, which acts on a substrate called angiotensinogen produced by the liver to form angiotensin-1. Angiotensin-1 is then cleaved to produce angiotensin-2 by an enzyme found mainly in the membrane lining of the lung. The drug, called a "converting enzyme inhibitor" prevents the enzyme's transformation of angiotensin-1 into angiotensin-2.

"This converting enzyme inhibitor acts in ways we don't understand very well", said Cantin. "Since the levels of renin in blood are not increased when the converting enzyme inhibitor is administered, and the levels of angiotensin-2 are probably not increased, the fact that blood pressure is lowered means that there must be some local renin-angiotensin system in the circulatory system that is very important in producing an increase in blood pressure, which is dampened by the converting enzyme inhibitor".

Cantin's group expects that the angiotensin-2 inhibitor will work in the same way in humans, and that it will decrease blood pressure even in hypertensives with normally high levels of circulating angiotensin-2 or renin activity.

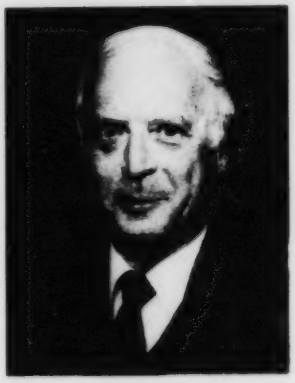
" We think more and more now that the cause of hypertension must be related to some effect of some hormones, probably in the blood vessels or in the heart or in the kidney... "

"We think more and more now that the cause of hypertension must be related to some effect of some hormones, probably in the blood vessels or in the heart or in the kidney, that we don't yet know about. I think the two biggest hopes for hypertension now are ANF, in whatever form, and this new angiotensin-2 antagonist".

The IRCM is probably the oldest centre for cardiovascular research in Canada: Dr. Jacques Genest, one of Canada's most distinguished medical researchers, started a hypertension group in 1952, which became part of the institute 20 years later, for the founding of which he was also responsible. This group, supported by MRC, "has made some of the most important contributions to the pathogenesis of hypertension in the past 30 years", wrote the American cardiologist, Irvine Page, in 1987. For example, Genest was the first to describe the disturbances of aldosterone in essential hypertension, and the role of angiotensin II in the control of aldosterone secretion, thus establishing the close relationship of the kidneys, the adrenal glands and sodium in hypertension. He has also made numerous contributions to the diagnosis and management of human hypertension.

It was Genest who brought Cantin to the IRCM as director of the pathology laboratory in 1982 (he became director of the hypertension group two years later). Recently, the scientific papers of Genest and Cantin were reported by the Philadelphia-based Institute for Scientific Information to be among the world's most-frequently cited in the 1980s. Genest's work ranked 53rd and Cantin's 62nd in this respect. Genest's work was cited 5,931 times and Cantin's 5,429 times in scientific journals worldwide.

As a boy, Cantin read much about Louis Pasteur's work, and decided while in university to become an MD and do research himself.



"I did a PhD in Montreal, then moved to Chicago and then returned to Canada", he said. "But I always did research. I never wanted to go into medical practice. I did a year of internship, but that was to help me in my research".

It's easier to do research now in Canada than it was then, Cantin thought.

"I was born in Quebec City and in the university there was not one professor or doctor who was interested in research. The first man of science I met who really was involved in research was Hans Selye. I did a PhD with him. He had a great influence on me: he showed me how to work in the lab, how to organize your life to be productive. He had his faults but he was a very efficient, well-organized gentleman and very imaginative. He was the first to show that you can produce hypertension with aldosterone in experimental animals, which I think was a tremendous discovery for the time".

In medical schools today, said Cantin, students are exposed to investigators who give courses and almost all professors in Canadian universities do at least some research. In Quebec, as in some other schools, at the end of the second year, students can spend a summer in the laboratory.

"In my time there was no such thing. I knew that the salaries for professors were so low that they had to do something else in addition to their work. This deterred me a little. Now the professors don't make as much money as if they were doing clinical medicine but at least they can live decently. And then there are many facilities for training, fellowships and so on".

Musing about his youthful attraction to science, Cantin recalled:

"I was always curious and I liked very much to work in the lab at school. I was happy there. It's still the place in which I feel I am most adquate. To tell you the truth, I never feel that I am working when I'm in the lab. I'm here all the time. I come here on weekends, too. I spend most of my life here, you know".

[1] Dr. Cantin died in June, 1990, of a heart attack. The remarks attributed to him here took place in an interview some weeks before.

"...I was always curious and I liked very much to work in the lab at school. I was happy there. It's still the place in which I feel I am most adequate. To tell you the truth, I never feel that I am working when I'm in the lab. I spend most of my life here, you know ..."

Dr. Geoffrey Flynn describes himself as an enzymologist-protein chemist who "didn't set out in life to do research that had a practical value" and who was simply "puttering away happily enough before I saw de Bold". Seeing de Bold changed all that. De Bold approached Flynn at a time when he was having difficulty getting a grant for his ANF research at Queen's University, and he needed a protein chemist to help him determine

the sequence of amino acids in the hormone's structure. "He couldn't get anyone interested in it", recalled Flynn. "It was the most amazing thing. People said, 'Oh, yes, you know, well maybe it's not a real result' (the demonstration of the atrial granules' diuretic activity). And there I felt a lot of sympathy for him. When he came to me, I can honestly tell you I thought it was the greatest thing I'd ever seen. I knew that this was something important, so I said, 'Well, I'll help you'. And we wrote a grant application. I went around this university borrowing money. I got a loan from the principal of Queen's University for $30,000 to do the research. I called on people I'd done favors for and said how much can you get me? We came up with about $50,000 and with that we bought thousands of rat atria and some

"We've said this has the potential not only to be a winner on the Nobel Prize market, but secondly it has the potential for being the anti-hypertensive drug of the century..."

equipment, and within a very short time we had purified and done all sorts of things with it – found its molecular weight and published that, and that started the ball rolling.

"We had to get money because there was commercial potential. We had to be the first to get it sequenced and fully characterized, so we assembled a team here of myself, de Bold, Peter Davies who was to do the molecular biology, and John Roder, who is now in Toronto, who was going to look at immunological aspects. That team put grant requests to IDEA Corp., a capital venture corporation that doesn't exist any more. IDEA Corp. went along and advanced us a lot of money. We put a strategic grant request to NSERC (Natural Sciences and Engineering Research Council), and NSERC pushed its strategic grant through very quickly.

"We had to get all this done rapidly. We went at it in a very business-like way, all of us here, and that's why the money came through. It was recognized by Canadians as being a Canadian discovery and a lot of people said 'Let's win something for a change'. The money came to well over half a million dollars, and we bought equipment and that allowed us to sequence and be the first to publish".

" I find it fascinating, and I am very interested in the application of molecular biology to the treatment of disease. I would like at the end of my scientific career to have made some contribution to medical science ..."

Of his part in the story, Flynn says: "If I give myself any credit at all it was for recognizing the problem as being of the magnitude it was, and I think it went speedily in the beginning because of that".

Flynn recalls that they were working in competition with people fro.. all over the world.

"It became a race to get the patent established and published. We published the sequence in December 1983, rapidly followed by everybody else".

"We were fortunate that there was a venture capital company available at the time. And we did a lot of persuading. Queen's University was very supportive at that point. IDEA Corporation had been set up by the Ontario government to support any venture that looked as if it might give employment opportunity to anybody in Ontario. We were the first to be financed by them. We said this has the potential not only to be a winner on the Nobel Prize market – and I still think that, if somebody finds out how it works, de Bold will still get a Nobel Prize because he discovered it – but secondly it has the potential for being the anti-hypertensive drug of the century".

Does he still think it has that potential?

"No, I don't. I think that something modelled on it might have, but it's going to take a long time to do that, because it's a peptide that is quite complex in its design and we don't know yet what the features of it which bind to the

receptors are. So it's going to take a long time, I think, before that's going to be worked out. But it's introduced a new concept into the field". Most of the research going on in the ANF field now is at the molecular biology level, said Flynn. The more recent efforts have come from the discovery of the new peptides in the ANF family: the so-called BNP (brain natriuretic peptide) and C-ANP or C-ANF (C-type atrial natriuretic peptide or factor).

"We've recently been involved in this, in that we've found what is the BNP peptide in rats, which we called iso-ANP, a name I don't think is going to stick, unfortunately. Despite the name, BNP is not a brain peptide primarily. There are vanishingly small amounts there, even though it was purified from brain".

BNP was obtained from the brain by Matsuo in Japan because he happened to have developed an extremely sensitive assay system for ANF based on the relaxation of chick rectal smooth muscle, said Flynn. "You've only got to look at chick rectal smooth muscle and it twitches. The rest of us were using an assay involving relaxation of rabbit smooth muscle, which was not terribly sensitive".

"Matsuo tried to purify ANF from brain and he did in fact succeed in doing that. In doing the purification he had noticed two or three other peaks of activity in the brain, and being an enterprising biochemist he went back and looked at them, and that's how he found BNP".

But it took 40 kilograms of brain from 500 pigs to isolate 10 micrograms of BNP, Flynn noted.

"That amount could possibly be accounted for by the peptide contained in blood from the brains rather than from the brain tissue itself. BNP originates in the heart – it's a cardiac peptide – and we have recently shown, though it's not published yet, that it's in the atrial granule with ANF. The question now is, what does it do?"

Flynn and his colleagues obtained the peptide in a different way.

"In purifying ANF we had noticed that what appeared to be a fragment of ANF – we weren't sure what it was – when sequenced did not give the same sequence as ANF. It did not give any portion of the same sequence, yet it had biological activity. I had a little argument over this with de Bold, actually: he said it was probably contaminated with ANF and we shouldn't investigate it any more. And in fact six months later he gave me a fraction that he said was ANF, and it was not, it was again this other substance.

"It was too much of a coincidence. So what we did was we chemically synthesized the unknown sequence that we had, injected that into rabbits, got an antibody, made a radioimmunoassay, went back into the purification system, and fished out this new peptide. Very much like Matsuo's twitch-thing, we used the RIA as an assay system in purifying that peptide from rat atria, and fished out this new substance, which we called iso-ANP and which Matsuo and others call rat BNP".

Flynn currently is working on the new atrial peptides. He has a grant from MRC with Stephen Pang, a young colleague in anatomy.

"We are looking at the molecular biology and cell biology of new atrial peptides like what we call iso-ANP", said Flynn. "The advances in this field recently have certainly come through molecular biology and biochemistry, not so much through physiology and pharmacology. But I'm biased: I'm a molecular biologist and biochemist".

Flynn's experience with ANF has caused him to modify his original outlook on research somewhat:

"I find it fascinating, and I am very interested in the application of molecular biology to the treatment of disease. I really do think that you shouldn't have that as a goal, but if it happens to come along in your research you should go after it in a whole-hearted way. I would like at the end of my scientific career to have made some contribution to medical science".

After John Ledsome had completed his medical training, he decided he didn't like the idea of practising medicine "not knowing what I was doing". By that he meant "handing out pills without really knowing why this pill was good for that person". So he accepted a job in the physiology department of his university

HEART MUSCLE STRETCH...

in Britain. He already had some practical experience in that field: having done his medical degree in Britain in the 1950s, he had to join the armed forces for two years, and he'd volunteered for the navy. Appointed to submarines, he learned a lot about physiology because the training school used decompression chambers, and since subs operate at different pressures, regular measurements had to be made of humidity and oxygen and carbon dioxide concentrations. "It impressed me with how little physiology I knew, and when I came out of the navy I decided to take a job in the physiology department for a year and learn a bit more". He was then attached to a senior department member to do research, who also had worked in submarines. Ledsome's mentor was "a great

"With ANF after 10 years we know as much as about most hormone systems, but it has been a bit more difficult to pin down whether or not it really has an important action."

enthusiast for research and taught me everything I know", and they both became excited about the work they we were doing on control of blood volume.

"...I think it's incredible the amount we've learned in 10 years: this is an area that has really shown the power of research. With ANF after 10 years we know as much as about most hormone systems..."

"Some people had just published a paper showing that if you distended the left atrium in dogs, they excreted more salt and water, and they claimed that was due to sensory nerve endings in the heart inhibiting release of vasopressin in the brain. We didn't believe it. However, it turned out to be true".

Ledsome and his research supervisor started working on this project, and later Ledsome went to the Cardiovascular Research Institute at the University of California at San Francisco for a year on a U.S. Public Health Service postdoctoral fellowship. He returned to Britain, as required by the fellowship regulations, but when back there he realized that medical practice was an unlikely alternative because he'd been away from it for so long. So he continued doing research with a clinical component in the physiology department. In 1968 he accepted a post at the University of British Columbia. When the expected opportunities for clinical research there did not materialize, he took over the pulmonary laboratory in the veterans' hospital, where he still works part-time.

When ANF was discovered, Ledsome had already been looking for such an agent for some 20 years. But as noted in the Introduction, he never expected it would originate in the heart.

"We couldn't explain why if you do things to animals such as increasing their blood volume or distending their atrium, you would get an increase in sodium excretion. It couldn't be explained by any known mechanism. It seemed to be related to distension of the heart.

"That was why I was interested in the nervous innervation of the heart and the sensory information that went to the brain. So we recorded the action potentials and studied the information that was going from the heart to the brain. We even blew balloons up and distended the atria. I well remember that one time we were using balloons that had talc on them from the fingers of our surgical gloves, and when we blew these balloons up in the atrium we got an increase in sodium excretion, and we thought it must be something coming off the balloons and getting into the blood. We said 'We've got to wash this talc off'. Even then it didn't occur to us that there was something coming out of the heart".

The ANF discovery was made just at the time that all the new biochemical techniques for isolation, purification and identification of substances had become available, said Ledsome. So in a very short period of time it was known exactly what the hormone was, people had synthesized it, and it could be measured in the plasma. Had it been discovered 20 years earlier, none of

that would have been possible because the techniques had not yet been developed.

Ledsome's current research has to do with control of the cardiovascular system – and particularly of blood volume – by two hormones: vasopressin, from the pituitary gland, and ANF, from the atrium.

For the cardiovascular system to function normally you need not only the blood vessels and the heart but a normal volume of blood inside them, said Ledsome. That volume doesn't change much from day to day.

"And any time we see some variable like that constant in the body, it usually means there are control systems. This is a fairly complex system that depends on intake of water and salt, distribution of fluid and electrolyte between cells and extra-vascular spaces and excretion of salts and water.

"The two hormones, vasopressin and ANF, have different actions and appear to have very different mechanisms controlling their release. Vasopressin controls water excretion. It is also a powerful constrictor of smooth muscle around blood vessels, so it can put up the blood pressure by constricting blood vessels. Thus abnormal secretion of vasopressin might play a role in high blood pressure.

"Vasopressin comes out of the brain and is controlled partly by the concentration of fluid in the body, to which it is very sensitive. But it is also controlled by nervous signals it gets from a whole variety of sensory nerve endings in the blood vessels, which sense arterial pressure, and also by sensory nerve endings in the heart. These are in the walls of the atrium and they send signals up the vagus nerves to the brain to influence the release of vasopressin.

"So this particular hormone is released in response to three things: changes in the concentration of the fluid, pressure in the arteries and the size of the heart. Any time the volume in the system increases, the amount of vasopressin being released is decreased, you excrete water and it brings the volume back to where it should be".

Scientists have known about vasopressin since the 30s, but until about 10 years ago they couldn't measure the concentration of the hormone in the blood. Their experiments to study vasopressin had to measure things like water excretion in the kidney, and from that they had to deduce what was happening to vasopressin. It's still difficult to measure, but about 15 years ago radioimmunoassays sensitive enough to measure vasopressin began to be developed. This meant that a lot of experiments done previously had to be repeated to confirm that in fact what they were looking at were changes in the concentration of vasopressin. Largely they turned out to be correct. But the

system turned out to be very sensitive to small changes in concentration of body fluids and to neural signals from the blood vessels and the heart.

"We still don't know whether excessive secretion of vasopressin plays any part in development of high blood pressure", said Ledsome. "But if you take groups of hypertensive patients, the majority appear to have normal circulating concentrations of vasopressin. There isn't a high vasopressin group of hypertensives as there is a high renin group of hypertensives, for instance. Nevertheless most people feel that vasopressin is such a powerful vasoconstrictor that it is a factor we have to consider in high blood pressure.

"Nor do we know in enough detail how the various stimuli to vasopressin interact. The problem of vasopressin and body fluids is that it only influences water excretion. If you were to eat a lot of salt, the fluid volume in your body would increase, but because its salt concentration would be high, that would be a signal for vasopressin not to excrete water. So unless you also have a mechanism for controlling salt concentration, vasopressin isn't a good system on its own. Which is why for 20 years we've been looking for a natriuretic hormone that would control salt excretion".

When de Bold discovered atrial natriuretic factor, it clearly was something very close to what Ledsome and others had been interested in all along: it was secreted from the heart, one of the major sites of nerve endings controlling blood volume, and it increased sodium excretion.

"However", said Ledsome, "we expected that it would be released in response to similar sorts of signals that affect the release of vasopressin, that is neural signals. It was a surprise to find that it appears to come out of the atrium in response to stretch of the atrial wall".

In one way this made sense: it meant that if blood volume increases, the heart gets bigger, ANF comes into the blood and promotes excretion of salt and brings the concentration back to normal.

"But what it doesn't seem to have is some other modulation of its release to balance it with other factors like arterial pressure or concentration of the fluids. I suppose we've got used to having some sort of central nervous control over these hormones, yet this one doesn't seem to have it. It seems to respond only to stretch of the atrium, and I suppose we're unwilling to accept that. So most of my research at the moment is trying to look at the mechanisms that release atrial natriuretic peptide with the hope that we will discover others that modulate its release".

Had the Ottawa ANF symposium – the summation of 10 years of research – given him any new clues?

"I think what it is showing is that the atrial peptide probably isn't just a sort of vestigial fluke that maybe somewhere in our past we needed, and it's still hanging around. It appears that the system is highly complex, with a whole series of different types of receptors and binding sites in important places in the body, most of which are concerned with fluid volume control. It really does look as if it is an important system which is influencing fluid exchange in many systems in the body, and probably is tied in with the central nervous control of other hormones like vasopressin and renin and angiotensin. But it's so complex that it's really going to take an awful lot of work to understand what it's doing. Some of the other hormone systems look quite a bit simpler".

Is it surprising that after 10 years we don't know any more about ANF?

"Oh, no. I think it's incredible the amount we've learned in 10 years: this is an area that has really shown the power of research nowadays. I think perhaps we expected too much – to understand it all. And we certainly never have with any of the other hormone systems that we've known about for a long, long time. There's still a lot of work to be done on insulin and its release and the ramifications of its actions. And that's after 70 years of work. With ANF after 10 years we know as much as about most hormone systems, but it's been a bit more difficult to pin down whether or not it really has an important action or not".

But Ledsome had one cavil:

"One of the striking things about this meeting is the large amount of work that's being done on such things as the molecular biology of receptors and binding sites, and the very small amount that's actually being done asking what this hormone does in the whole animal, and what the control mechanisms are. That's a bit worrying, because we're not getting the whole picture. It's a bit unbalanced".

"We may never completely understand it, but that doesn't daunt me. That excites me, that the system is so complicated and so interesting. That is why I'm in this business"

Medical scientists like to quote a truism about research: each new discovery provides more questions than answers. Nowhere is this more true than in cardiovascular research. The human cardiovascular system is one of incredible complexity, and with each new discovery the complexity seems to increase. Some would find this daunting. Dr. Andrew Rankin of Memorial University of Newfoundland, finds it stimulating. "We may never completely understand it", he said, "but that doesn't daunt me. That excites me, that the system is so complicated and interesting. That's why I'm in this business. We're not in it for the money, as you know. The only thing one gets daunted by sometimes is making out grant applications – selling one's ideas. And, of course, you sometimes run into dead ends". What appealed most to Rankin about de Bold's ANF discovery was that it upset classical ideas about cardiac physiology held for centuries. One aspect of classical physiology currently being challenged in Rankin's mind has to do with the interactions between the cardiovascular system and the kidneys.

"It's been known for a long time that the central nervous system innervates the kidneys through nerves going *to* the kidneys", said Rankin. "These control the blood vessels in the kidneys by constricting them and changing the kidney's filtration of body fluids: they can produce renin and spark the renin-angiotensin-aldosterone hormonal system into action. These nerves are called *efferent* nerves.

"We've also come to understand that there are nerves going *from* the kidney into the central nervous system, called *afferent* fibres. This is very much a Canadian project: John Ciriello, a neurophysiologist, and his colleagues at the University of Western Ontario (UWO) have traced the pathways and located some of the areas of the brain to which these fibres are connected. These areas are important in cardiovascular control – the medulla oblongata and also the hypothalamus. He's made a connection between these afferent fibres and the release of vasopressin, another peptide I've been interested in the past. It's not clear what this system is doing.

"We also know from earlier studies by another UWO researcher, Franco Calaresu, that if you stimulate these afferent fibres electrically, you can get changes in blood pressure and heart rate. No more has been done with it. It's very difficult to stimulate these nerve fibres selectively to find out what the physiological stimulus may be. Are the afferent fibres connected to pressure receptors? Obviously there are connections with vasopressin, which acts on the kidney and the circulation. Are they responding to changes in blood chemistry, for example hypoxia (lack of oxygen)? Probably. Are they innervating the other side of the kidney? If you change the ionic environment of the kidney, you get firing of these fibres as well.

"What we're trying to do – and it's not an easy project – is to see what the stimulus is, or what the stimuli are, because maybe there are many stimuli. And what the resulting cardiovascular and renal changes might be.

"It's becoming clear that not only are there reflex systems we've known about for decades, but there are also afferent fibres from many other organ systems that have an effect on circulation. If you look at the gut, there are afferent fibres there – perhaps also in the spleen, all sorts of places. Lynn Weaver at UWO has done some work on visceral afferent nerves that control circulation".

These complications to the system of cardiovascular control have been apparent for 20 years, but they've been largely ignored, said Rankin.

"My suspicion is that there are all sorts of inputs to the cardiovascular control areas of the brain, and we know about many of the major inputs, but we

don't know much about these subtle influences. So rather than sorting out all the pathways of cardiovascular control, we're turning over new stones all the time".

No one knows how important are what Rankin calls "these subtle influences". But, he asks rhetorically:

"What if they are incredibly important? And for goodness' sake we've already got so many links between the cardiovascular system and the kidney – from renin, angiotensin, aldosterone, ANF and vasopressin. Perhaps there are other neural reflexes and hormones involved; it's an incredibly complicated mechanism".

Rankin first came to Canada from England in 1983 as a Canadian Heart Foundation Fellow to work with John Ledsome at the University of British Columbia on mechanisms of release of vasopressin.

Recalling how he got into research, he said:

"I can remember playing around with batteries and light bulbs and things. I wasn't particularly good at it. I remember in high school I found the body and organisms very interesting and exciting. My older brother went into agriculture: both sides of the family were farmers originally. My father is now an engineer, so maybe he encouraged our scientific interest a little.

"My suspicion is that there are all sorts of inputs to the cardiovascular control areas of the brain, and we know about many of the major inputs, but we don't know much about these subtle influences."

"I liked working outdoors, so I started doing a fairly broad biology degree with the idea of deciding whether I wanted to go into the ecological side or the life sciences. I did an applied biology degree, and after two years you could either go out into industry or carry on with the final stages of a degree. I still wanted to go out and see what industry was like and by that stage, I'd decided that the life sciences were more interesting. So I went to work for Beecham Pharmaceuticals for a year. And that was a tremendously valuable experience. The main thing it showed me was that I needed a higher degree.

"I didn't know what a PhD was at that point, and I don't think most school kids know today: they don't know what the career structure of science is".

Rankin believes scientists like himself should be out talking to students in the schools, and he has made himself available to the university speaker's bureau, though he's not sure that is the best vehicle.

He started graduate work on receptor binding in a cardiovascular pharmacology project, but to his dismay found it boring. At that point he "made possibly the most difficult decision I'd ever made and that was to switch to another university and try to convince someone to give me a more physiological project".

He was in the University of Wales in Cardiff, and went to the University of Leeds on a British Heart Foundation studentship. Working with Dr. Roger Hainsworth, he spent just over three years there and got a PhD in cardiovascular physiology.

"I never made a conscious career decision", said Rankin. "I wouldn't have done this if I had: I would have gone into medicine. You only find the people who really enjoy it staying with it, because the tangible rewards are so few and the difficulties are so great. Even if you obtain a PhD, normally you then do at least one post-doc; then you've got to get a faculty position if you want to go into academia, which are few and far between; and then you've got to get funded and stay funded, which is really tough. If you spell all that out to somebody, and tell them the salary at the end – what you expect to make 20 years down the road – they're not going to go into it".

That being the case, what keeps him in research?

"We have a certain flexibility as academics, and this is what I love about it. I've got to sell my ideas to the granting body, but I can go off in whatever direction I find interesting, within limits. What other job can you do that in?"

"We have a certain flexibility as academics, and this is what I love about it. I've got to sell my ideas to the granting body, but I can go off in whatever direction I find interesting, within limits. What other job can you do that in?"

"I often think that I would like 5 dollars from every surgeon this work has allowed to stay in bed, rather than having to get up in the middle of the night to go and operate..."

"The first patient we treated – and I think it was the first in the world – was my most dramatic night in medicine. This was a little baby who came in with a condition called pulmonary atresia, which is one of the causes of blue babies, in whom there is severe cyanosis. In those days – it was 1970 –we didn't have cardiac ultrasound available, so the only way you could make the diagnosis was to take the baby to the cardio-catheterization laboratory. By the time we did so, the baby was extremely sick: he was blue, limp and obviously dying in front of us, which was common in those days. We started this infusion of prostaglandins and in five minutes this baby had been transformed into a pink, alert, reactive baby. It really was incredible". During the late 1960s and early 1970s, Drs. Peter Olley and Flavio Coceani at the Hospital for Sick Children in Toronto became intrigued by the possibility that prostaglandins might be of use in the immediate treatment of certain congenital heart malformations in babies. Prostaglandins had in 1930 been isolated from the seminal fluids of sheep (hence the name, derived from that of the male prostate gland).

CONTROL OF THE HEART

"...The whole area of understanding the genetics of heart malformations is really a major one, and there isn't a lot of research going on in that area because it is difficult to do and there are not all that many people interested..."

Because of the multitude of metabolic effects they produced, they had become by 1968 the subject of extensive research.

"Almost all major structural defects of the heart have very little effect during fetal life", said Olley. "It's only after birth when the transition from the fetal circulation to the post-natal arrangement takes place, a number of special fetal channels close off and the lung circulation relaxes, it's only then that they begin to feel the full adverse effects of the heart defect.

"So our thought was, if we could arrest and maintain some of the features of the fetal circulation, that might give us an opportunity to intervene surgically more effectively".

Olley and Coceani were particularly interested in a blood vessel called the ductus arteriosus. This vessel, one of the biggest in the fetal circulation, closes off just after birth. "The problem for some babies is that the ductus arteriosus is the only route by which blood can get to the lungs because of other defects, or in other cases the only route by which blood can get to the systemic circulation. The treatment in a lot of these cases prior to this was to create an artificial communication which served like the ductus, but by the time you took the babies to the operating room they were very sick, and so there was a high mortality".

Olley and Coceani thought that, if they could find a way of keeping the ductus arteriosus open for a time after birth, they could stabilize the babies' condition and keep them in good shape physically until they could be operated upon to correct their congenital abnormalities.

"We knew from work done in England in the 1940s that oxygen was the main trigger to the closure of the ductus arteriosus", said Olley. "But nobody knew exactly how oxygen affected the smooth muscle of this blood vessel. Various chemical agents like adrenalin and acetylcholine and bradykinin had been looked at and didn't seem to fit the facts. We became intrigued by the possibility that prostaglandins might be involved".

Prostaglandins did not appear to be involved directly in the closure of the ductus arteriosus, but there was strong evidence that they were involved in keeping it open in the fetus. "This was a fairly revolutionary concept, that you might have a state of active maintenance of patency".

The first use of prostaglandins for this purpose, described above, showed that they could, indeed, keep the ductus open in babies with heart disease.

"What using prostaglandins did was to allow us to stabilize the baby, reverse all the metabolic effects of severe heart failure or severe cyanosis, and then take it to the operating room selectively, 24 or 48 hours or in some cases a

few weeks later, and be able to operate on a much healthier, stable baby", said Olley. "I often think I would like $5 from every surgeon this work has allowed to stay in bed overnight, rather than having to get up in the middle of the night to go and operate".

The ductus arteriosus can be kept open indefinitely in this way, but the time is limited in practice by the fact that the prostaglandins have to be given intravenously.

"This was a direct and very dramatic clinical application of something that had started off as a piece of basic research", said Olley. "The other side of this was that it led to the ability to close the ductus in premature infants, where it often stays open and creates serious circulatory problems. By giving them drugs that prevent the formation of prostaglandins you can induce closure of the ductus. That's again also become a standard treatment, usually using indomethacin".

Olley and Coceani were not sure which of the prostaglandins was the really important one: prostaglandin E-2 or prostaglandin I-2, or prostacyclin. There is a family of 20 or more related compounds, not all called prostaglandins but all derived from the same precursor.

"I had the idea that if we looked for specific protein receptors for one or other of these candidate prostaglandins in the smooth muscle of the ductus, that might give us a clue as to which was the really important one", said Olley. "But the practical problem was that, while the ductus is quite a big blood vessel, it's very difficult to get enough tissue from it by the time you've purified it and separated out the membranes. So I decided to start with the heart, because we could get beef hearts: they're big and there's lots of tissue and we already were separating out the membranes for other experiments. What we discovered was that there is a specific cell surface receptor for prostaglandin E-2 present in heart muscle. We've subsequently characterized that and purified it, and we currently believe it may be a very important regulator of the heart's response to adrenalin and noradrenaline particularly. We think it's a sort of fine-tuning regulator, not the primary regulator, but it fine-tunes the response: heart rate, force of contraction, the way in which electrical impulses are transmitted through heart muscle and so on".

Olley said there's a great deal of overlap in the control of the coronary circulation. There's still a lot of interest in the possibility that other members of the prostaglandin family – not E-2 – may be responsible for coronary artery spasm, and therefore heart attacks. The whole question remains open of how

these lipids influence the heart, and how they may be involved in the extent of damage after a myocardial infarct and in recovery after re-perfusion.

"There are specific drugs that will selectively inhibit one or other of the metabolic pathways of the precursor of all these prostaglandins, which may go in three or four different directions", said Olley. "If you can block the unfavourable direction then you may encourage the formation of the favourable compounds. There's been a whole host of experimental drugs, and they are beginning to become commercially available. There isn't one ready for large-scale clinical trials, but there's a great deal of interest in them. The approaches being taken are that either you can block prostaglandin synthesis, or if we understand the structure of the receptor we may be able to tailor-make drugs that will either block that receptor or stimulate it. So understanding the receptor would be a major conceptual step forward. At this university we have a very strong protein chemistry group. If we can work out the structure of the binding site then we'd be in a position to design drugs or chemicals that will also bind or block binding at that site".

Olley said the concept of receptors has been around a long time, but it's really in the last 15 years – or even more in the last decade – that the whole field has taken off. The big stimulus was first of all the ability to separate out the various components of the cell membranes; the availability of radioactively-labelled compounds; the advances in protein chemistry and the ability to separate proteins and handle very small amounts, and all the advances in molecular biology. Olley's work in this field began about three and a half years ago.

"We're at the stage where we've purified this particular protein. We're attempting to purify sufficient to be able to apply molecular biological techniques. The plan would be probably to try to raise antibodies to the protein, possibly to clone it. We're also doing a lot of work on signal transduction across the heart muscle cell.

"What we're looking at now is the relationship between binding of prostaglandin to this protein which is in the cell membrane, and how it influences the control of chemical change within the cell, and also functional change – heart rate, contractility, etc."

When Olley started working on prostaglandins with Coceani in the late 60s there were just a few other people in the whole world doing this.

"You could count them almost on one hand. The whole thing has since exploded. About ten years ago it really looked very much as though we might be looking at a major control system for the blood vessels in which the

whole integrity of the vascular system depended on a balance between thromboxane, which is a prostaglandin-like substance that comes largely from platelets and tends to constrict blood vessels and help blood clot, and prostacyclin, which comes largely from the endothelium, the lining cells of blood vessels, and tends to relax them and stop blood clotting. I think we've backed off a little from that. There was tremendous enthusiasm and this was when the pharmaceutical companies suddenly saw an opportunity".

One outcome was the proposal that taking an aspirin a day might protect against heart attack or stroke, because aspirin blocks the formation of thromboxane. A number of clinical trials resulted.

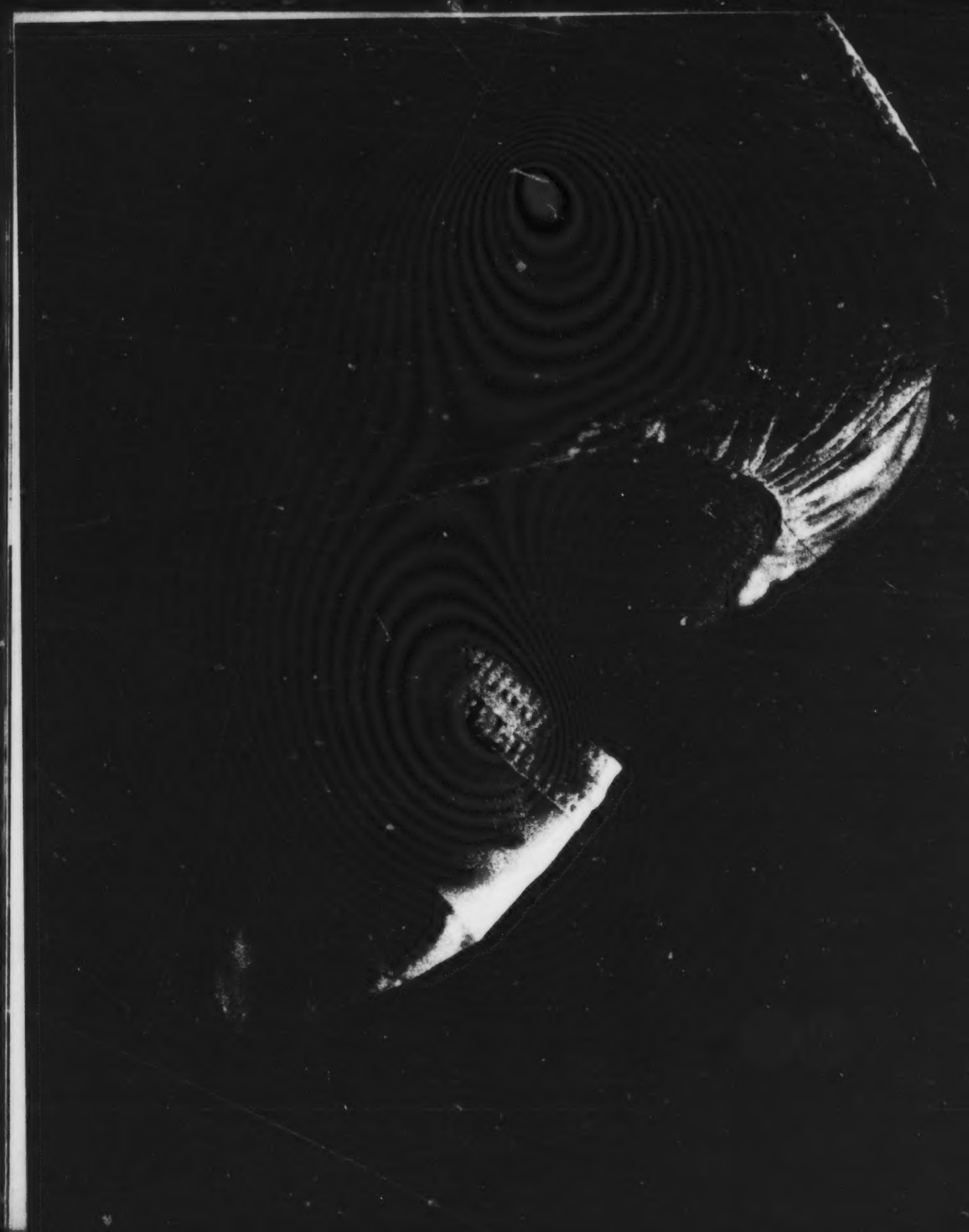
"The balance of evidence at the moment seems to suggest that an aspirin a day or a very low dose of aspirin may have a beneficial effect in preventing heart attacks", said Olley. "The trouble is of course that there are risks in taking aspirin continuously, particularly gastric ulceration. There's still a lot of uncertainty and controversy. But there are one or two trials where it's a little difficult to get away from the conclusion that in healthy males there does seem to be a beneficial effect from low-dose aspirin".

Olley originally got into research after training as a cardiologist in Britain.

"I started off in adult cardiology and used to look after patients with coronary artery disease and high blood pressure and so on, and then I got very interested in the problem of heart disease in children. That was what brought me to Canada. It really was this question of what could we do to delay the transition in patients from before birth to after birth as far as the circulation was concerned because this clearly would have a beneficial effect. So it really was curiosity about a practical problem that took me into research".

He says congenital heart malformations have become more important in Canada as other forms of cardiovascular problems have been reduced.

"If you look at morbidity or mortality in adult life, most of the mortality from congenital heart disease occurs in childhood. On the other hand if you look at 'useful years of life lost', congenital heart malformations become really quite significant. So the whole area of understanding the genetics of heart malformations is really a major one, and there isn't a lot of research going on in that area because it is difficult to do and there are not all that many people interested".



THE QUEBEC BEER DRINKERS' CARDIOMYOPATHY

In 1967, Dr. Yves Morin, cardiologist at Hôtel-Dieu Hospital in Quebec City – the oldest continuously functioning hospital in North America – noticed a new phenomenon. "We suddenly had patients coming into emergency who died in heart failure", he recalled recently. "They had a peculiar appearance to their heart muscle tissue at autopsy: vacuoles (cavities in the protoplasm), which was very unusual. They also had a strange skin color, and they died suddenly". All were men between 40 and 60 years of age. Many did hard physical work, such as stevedoring, and at first Morin and his colleagues thought the condition might be related to their occupations. But later they found many were unemployed. And then they found they all had one thing in common. "They all drank a lot of beer", said Morin. "The average was about 24 small bottles of beer a day. But we had a truck driver who drank 30 large bottles of beer daily while driving a truck between Montreal and Sept Iles. He kept a pail between his legs in the truck to pee in because he drank so much beer. He'd put a newspaper around the bottle so he wouldn't be seen to be drinking".

"They all drank a lot of beer. The average was about twenty-four small bottles of beer a day. We had a truck driver who drank thirty large bottles of beer daily..."

Because of this, Morin thought what they must be suffering from was a condition known as alcoholic cardiomyopathy. But Hôtel-Dieu Hospital was fortunate at that time in having an excellent pathologist on staff, Dr. Jean-Louis Bonenfant, who insisted that what he saw in his microscopic slides of the victims' heart tissue indicated something quite different. Also, the new disease was fulminating – it came on suddenly with intense severity – while alcoholic cardiomyopathy has a slow, extended onset.

"It's a different clinical picture", said Morin. "There's no clustering as there was here in Quebec. Some died in emergency and there was a 50% mortality, which isn't the case with alcoholic cardiomyopathy".

Morin and his colleagues knew they were really on to something when questioning of the patients revealed that they all drank the same brand of beer: Dow.

"We would never have discovered what it was had we not had a situation in which there was a local brewery in Quebec City that manufactured Dow, the leading brand at the time", said Morin. "They brewed only for a small area centred on Quebec City, and a market survey had shown that people there liked beer with a head on it. The taverns washed the glasses by putting them in a detergent solution and didn't rinse or dry them. So there was a film of detergent that stayed on the glasses which prevented the head of foam from forming. To counteract this, the brewery had added an anti-detergent to the beer – and that anti-detergent was cobalt".

Like modern-day Sherlock Holmeses, the Hôtel-Dieu staff demonstrated that the cause of the new heart condition was the cobalt. For although cobalt was added to beer elsewhere – for example, in Montreal, the U.S.A. and Belgium – the amount added in Quebec City was extremely high – about 1,000-fold more than in Montreal. Furthermore, experiments the medical team undertook demonstrated the same sort of lesion could be produced in guinea pigs.

Despite the skepticism of the beer company, which at first kept insisting that the disease was simply alcoholic cardiomyopathy, Morin and his associates had proved their point.

"We invented a new disease", said Morin. "It became known as Quebec Beer Drinkers' Cardiomyopathy. It lasted for maybe a year and then it disappeared".

The reason for its disappearance was that the cobalt additive was withdrawn, not only by Dow (which later went out of business), but throughout the world. Part of Dow's problem had been that, unlike other breweries, it used the

same beer in draft form and in bottles. That fact, however, made the cause easier to find because it increased the clustering effect.

After the story appeared in the press, the Hôtel-Dieu group received a call from Omaha, Nebraska, where a similar situation seemed to be developing.

"I flew there with a pathologist", said Morin, "and found everybody afraid of being sued. The evening we arrived we said, 'Well, let's go and have a drink'. We had beer and they said, 'We have one local brewery that makes the beer', and I asked 'Which brewery is it?' And I remember the fellow didn't dare say the name – he pointed at the beer I was drinking! Stohrs. The Stohrs family was extremely powerful in Omaha and everyone was worried".

Morin saw cases there exactly like those in Quebec. He brought back some bottles for a biochemist, Dr. Guy Nadeau, who was doing the cobalt measurements.

"It was the same story: a small brewery making beer both as draft for the taverns and for bottling", said Morin. "You see in Montreal they did add cobalt but there was so little of it, only in taverns. Nobody added cobalt to bottled beer because the detergent problem wasn't there. But a small brewery could not make different brews for draft and bottling.

"...So there was a film of detergent that stayed on the glasses which prevented the head of foam from forming. To counteract this, the brewery had added an anti-detergent to the beer – and that anti-detergent was cobalt".

"Then we found another place with similar problems in Minneapolis, and there were places in Belgium, where they have hundreds of small breweries. Finally they closed the brewery here, which was a shame in a way – it was the oldest in North America – and there was a lot of resentment against us at the time locally. But all they would have had to do was take the cobalt out".

The episode made Morin and his colleagues world-famous as cardiomyopathy experts: in 1967 a special issue of the *Canadian Medical Association Journal* was devoted to the new disease, and medical journal articles appeared around the world. But it also added to the store of knowledge about this form of heart disease. And it helped protect consumers from possible repetitions of similar risks.

"We didn't realize at that time that cardiomyopathies could be fulminating and have patients die within a week", said Morin. "This was shown clinically to be so. Also, until then there had been no tests done for the effects of additives on the heart. Now every time a new additive is going to be used in something people will consume, an animal study is done on the myocardium. The Health Protection Branch of Health and Welfare Canada told me it's surprising how many agents are toxic for the myocardium. We now realize that the heart is far more sensitive to various agents, both toxic and infectious, than we thought it was before".

Morin took his medical training at the University of Toronto and Toronto General Hospital (TGH), which was unusual for a French-Canadian in those years (1956-62). Aside from Morin's superior abilities in English, this was made possible by Dr. Ray Farquharson of the TGH, who initiated the practice of bringing French-speaking Canadians to Toronto. Farquharson also played a large part in the establishment of the Medical Research Council of Canada and was its first president.

In Toronto, Morin came under the influence of Dr. Douglas Wigle, an expert in cardiomyopathy, who arranged for him to go to Great Britain's National Heart Hospital to study under the Director, Dr. Paul Wood. Morin says Wood influenced not just himself but a whole generation of cardiologists in the U.S.A. and Europe.

Unlike some of the other researchers interviewed for this book, Morin did not always have a strong interest in science as a youngster. Much less research was being done in Canada when he was at medical school, and though he was interested in the heart in a general way, it was more the influence of individuals like Wigle and Wood that guided him into his life's work.

"...Finally they closed the brewery here, which was a shame in a way – it was the oldest in North America – and there was a lot of resentment against us at the time locally. But all they would have had to do was take the cobalt out..."

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" The body controls blood volume very closely. If there is too little, the animal becomes thirsty, and produces less urine. If there is too much blood, more urine is produced. However, this is not all. There is evidence that fluid may be redistributed within the body, i.e. there are reservoirs where fluid may be stored in time of plenty and drawn upon in time of need. I propose that, when the heart detects that there is a fluid surfeit, it sends out nervous and hormonal signals, not only to increase urine output, but also to reduce fluid uptake from the gut and to increase the filtration of cell-free fluid through the spleen and into the lymphatic system. I will investigate, in the rat, the effects of fluid loading, of haemorrhage and of hormonal manipulation, on salt and water movement across the gut wall and through the spleen into the lymphatic system. Using a tiny balloon implanted in the heart, I will stretch the heart, thus mimicking the conditions of fluid surfeit. It will then be possible to study how signals from the heart adjust fluid balance. Finally, using a

LIZARDS, LYMPH SACS AND BALLOONS...

"...It's the very first time that the spleen has been shown to have a role in regulating blood volume, apart from acting as a reservoir for holding whole blood..."

lizard that stores its spare water in sacs under the skin, I will seek to understand how lymphatic fluid volume is regulated".

Thus, in a model of clarity in plain language, did Dr. Susan Kaufman of the University of Alberta's Department of Medicine summarize one of the projects for which she was applying for a research grant from the Medical Research Council of Canada.

After the work was underway, she expanded on her progress: "We've developed the system where we put a little balloon in the heart of the rat. We've shown that when we blow the balloon up it increases the urine output: the rats drink less and take in less salt. What the balloon is doing is simulating the presence of more blood in the body because it's stretching the heart muscle. We've already shown what goes in and what comes out: now we want to show what goes on in the middle".

"I really wanted to do something that will lead to a much more basic understanding that has some application ultimately to humans and to medical research..."

One of the compartments in the body in which fluid can be stored is the gut.

"We did some preliminary work with that in which we were able to show grossly that the state of dehydration of the animal affected the amount of fluid held in the gut as a reservoir. So if the animal was dehydrated it needed to pull more fluid out: it was very simple, but no one had shown it. Now we want to go on to see where the signals are coming from that tell the body that there is not enough water in it, and what the mechanisms are that control the movement of fluid out of the gut".

The spleen is one of the organs that acts as a reservoir of blood in some animals such as the horse and the dog. For example, in case of shock, as in an accident with loss of blood, the spleen contracts and forces out blood it is holding into the circulation. Such an effect, which occurs in addition to the spleen's other functions, is not very large in man, and in rats it is even smaller.

"What I proposed was that the spleen may be a gateway to control the movement of fluid from inside the blood vessels into the lymphatic system. The lymphatic system collects the cell-free fluid leaking out of all the blood vessels, pumps it through a central conduit, and eventually dumps it back into the heart.

"The plumbing of the lymphatic system can hold less or more, depending on the state of hydration of the animal, and it's very hard to get at this in mammals because it's a very diffuse system. We wanted to see whether, through the spleen, fluid was shunted out into the lymphatic system – and whether, if we volume-loaded the animal, we would then increase the amount of fluid going out. In other words, you put extra volume into the blood. Now there's

too much there. How can it get rid of it? It shunts it through the spleen, dumps it out into the lymphatic system".

As expected, Kaufman and her summer student, Matthew Choi, found it technically rather difficult to measure what was going on because the blood vessels involved are small. But they succeeded in cannulating the blood vessels draining the spleen with the aid of tiny tubes, and measuring the thickness of the blood and the amount of protein in it.

"And indeed, we do find that if we volume-expand the rats by injecting saline into the circulation intravenously, there is an immediate rise in protein content and increased thickening of the blood leaving the spleen as compared with that going in. That means that fluid is being filtered out of it, and this seems to be under some sort of control.

"It's the first time the spleen has been shown to have a role in regulating blood volume, apart from acting as a reservoir holding whole blood. That's different because in that case the spleen holds whole blood and just squeezes it out.

"We would like to be able to show that if we caused haemorrhage in the rats there may even be a net movement of fluid in. We don't know. We've got to look at that, with this idea of its being a gateway of fluid in and out of the circulation. Having established that there's obviously something going on there, what's controlling it? We can look at neural control – there are plenty of nerves to the spleen – and see what effects they have. For example if we cut those nerves, can it still do it? Or is it a hydrostatic mechanism dependent on pressure? Is it hormonal? We can look now into hormones we are familiar with, such as angiotensin and ANF".

One of Kaufman's helpers in determining how lymphatic fluid volume is regulated is a lizard called a chuckwalla that lives in southern U.S. deserts. When it rains, this little animal stores fluid in lymph sacs that line the side of its body, and when the weather is dry these sacs shrink.

"It's lymph in there", said Kaufman. "So what we want to do is to get some of these animals and see what effect ANF has on this process: does ANF influence the volume of lymph stored in the lymphatic system?"

Kaufman plans to measure the amount of fluid in the sacs before and after administering ANF to the animal, to see if the amount changes. She has already worked with iguanas at Cambridge University, and says they withstand surgery well. As for the lizards:

"We've tried withdrawing the fluid: it's dead easy, you just put a needle in and draw it out, measure how much is in, put it back in again under sterile

conditions and give the ANF and measure to see if it's changed. Eventually it would be very nice to be able to look at some other things, such as stretching the heart, and see whether it has the same effects".

Although Kaufman's work is extremely basic in nature – trying to determine the nature of a physiological mechanism – it obviously has practical implications. For if fluid balance in the body can be better understood, this knowledge could be put to work in solving cardiovascular problems.

An even more direct practical application is apparent in Kaufman's other work on blood pressure and volume in pregnancy.

"There's a whole slew of experiments that need urgently to be done", she said. "Normally during pregnancy there is a drop in blood pressure and the pressor response to injected angiotensin is reduced. In toxaemia, which is still a terrible problem, the patient becomes hypertensive. Interestingly, when you get toxaemia, the blood pressure response to angiotensin returns to that of the non-pregnant state even before the blood pressure goes up – even before they become toxic.

"And so we have to understand what's happening here. The question also has arisen with toxaemic patients, do you reduce or increase their salt intake? This is not really known. So one of the things we're looking at is the effect of salt intake on the pressor response to angiotensin during pregnancy. We've been feeding the rats a low salt diet and a high salt diet and we find totally different responses. Essentially, with a high salt diet we retain the pressor responsiveness throughout pregnancy whereas with a low salt diet the response is progressively reduced. These are, of course, preliminary results, but they may prove to be significant in the management of hypertensive pregnancies".

Kaufman is a chemistry graduate who first decided that, because "I'd always been interested in creepy-crawlies", she was going to combine her interests in biology with those in chemistry by looking at the effects of toxic chemicals on the environment and pollution.

"That was in 1965, long before it became fashionable, and I've wondered sometimes if I shouldn't have gone into that. Anyway I switched to doing a master's in zoology, and one of the courses I took was insect physiology – the first physiology I'd ever taken in my life – and I was so interested in it I abandoned all my chemistry and went into physiology".

She had found zoology rather disappointing, partly because there was a lot that was purely descriptive, and she was much more interested in mechanisms. She found she could not justify to herself spending money and time simply on describing animal systems.

"I really wanted to do something that will lead to a much more basic understanding that has some application ultimately to humans and to medical research. I did my undergraduate work in London and graduate work at McGill and UBC and then went back to Cambridge to do a post-doctoral and then to Switzerland for three years. In Cambridge I worked with James Fitzsimons, who is the granddaddy of the physiology of thirst – the control of fluid and salt intake – and continued that work in Switzerland with George Peters".

Kaufman explains that her interest in the lizards is not just professional:

"I like reptiles. I'm very fond of them, and I've kept lots of them for a long time. I've had one at home for about 13 years. The chuckwalla in the lab is named Pancake and the one at home is Chumpo. He's very big and very fat".

"We feel that in probably as many as 40% of patients suffering from essential hypertension, the sympathetic system may be implicated in development of high blood pressure..."

The more we learn about the mechanisms that control blood pressure in the human body, the more complex they appear. In a healthy individual, these mechanisms work together in a marvellously collaborative fashion to ensure that blood pressure remains within what physicians deem normal limits. Wrote William Boyd: "In spite of the fact that these varied factors are continually altering, so perfect is the co-ordination between the several parts of the circulatory system that under ordinary conditions any sudden change in pressure is rapidly restored to normal. Should the pressure be continuously below or above normal the condition is called respectively hypo- or hypertension". Two obvious pressure control mechanisms in this intricate plumbing system are the contractions of the heart – the pump – and the resistance offered by the blood vessels – the pipes. But scores of others are turning up, as we have seen in earlier chapters dealing with ANF. In recent years, Dr. Jacques de Champlain and his associates at the Research Centre of Sacré-Coeur Hospital in Montreal, have discovered yet another influence that applies in certain cases: the sympathetic nervous system.

SETTING THE THERMOSTAT TOO HIGH

The sympathetic nervous system consists of the fibres and nerves that supply structures such as the alimentary canal, blood vessels, lungs, heart, uterus, glands and the urinary bladder. Its actions are not subject to voluntary control and are for the most part unconscious.

"We feel that in probably as many as 40% of patients suffering from essential hypertension, the sympathetic system may be implicated in development and/or maintenance of high blood pressure", said de Champlain.

Hypertension appears in what have been designated as two major forms: primary or essential, where the cause is unknown, and secondary, where it results from some associated condition such as inflammation of the kidney. The term "essential hypertension" is actually meaningless, and simply indicates, as Boyd said, "our lack of any real knowledge of the nature and cause of the condition". Presumably whoever made it up considered that it must be "essential" because it is there! What is important is that the great majority of cases of hypertension – an estimated 90% – fall into this category.

Despite this importance, which follows from evidence that those with hypertension are at greater risk for heart attacks and strokes, physicians disagree on what defines essential hypertension – that is, what should be considered the normal range of blood pressure. The usual definition is that it is represented by sustained blood pressure higher than 150 mm of mercury systolic and 90 mm diastolic. On the basis of some studies, that would suggest that between 10 and 20% of the population of Canada would be so classified. However this may be, de Champlain's discovery can be seen to be significant if only because it has serious implications for a large number of people.

De Champlain said it has become known that the sympathetic system can be a factor influencing excessive growth of cardiovascular tissues. This could lead to shrinkage of the blood vessel lumen and enlargement of the heart – complications of hypertension that eventually produce heart failure or coronary heart disease.

"That's why we're so interested in the sympathetic system, because we think its hyperactivity may constitute what you'd call an additional risk factor", said de Champlain.

De Champlain determines whether or not a patient's sympathetic nervous system is hyperactive by measuring neurotransmitters circulating in the blood. Neurotransmitters are the means by which the nervous impulses of the sympathetic system are communicated to their target cells. They include catecholamines (epinephrine and norepinephrine) and neuropeptide Y. Newer,

more sensitive measurement methods have made it possible to learn what is going on in the sympathetic nervous system in this way.

In 1983, an investigator named D.S. Goldstein reviewed the world literature on circulating catecholamines in hypertensive patients and concluded from a large number of studies that a subpopulation of hypertensive patients exists who have higher-than-normal levels of circulating catecholamine. De Champlain found that, comparing such a group with those with normal levels of catecholamine, the former also had a higher heart rate and increased contractility of the heart muscle. It thus seemed that, in some patients, an increase in sympathetic nervous system activity might be responsible for the higher heart rate and muscle contractility.

"...After completing my PhD in clinical research, I wanted to understand basic mechanisms and so decided to go into basic science for three years of postdoctoral studies..."

De Champlain also found that when the group with higher levels of catecholamine had their blood pressure taken while standing for 10 minutes, the majority also showed greater increases of norepinephrine levels than the other group. Since postural change activates the sympathetic system, this suggested that the high catecholamine group had a system that was hyperreactive.

Exercise also elevates the blood levels of norepinephrine, probably through a mechanism similar to that found in postural change, suggests de Champlain's group. They noted that, in a group of hypertensive subjects with normal catecholamine levels who exercised for one hour on a bicycle ergometer, both norepinephrine and epinephrine levels and blood pressure rose more than among non-hypertensives. These levels also took a longer time to return to normal than in non-hypertensives, suggesting that something was wrong with the mechanism that lowers sympathetic system activity.

The response of the two groups of hypertensive patients to drugs known as beta blockers, which are used to lower blood pressure, also differed: the patients with hyperactive sympathetic systems responded far better to these drugs. Their heart rate and heart muscle contractility also were more reduced. At the same time, these responses were associated with reductions in norepinephrine levels when they stood or lay down.

As if this picture were not complicated enough, de Champlain notes recent findings which have helped to refine our understanding of the cellular reactivity to the activation of the sympathetic system:

"It has been found there are co-transmitters, such as neuropeptide Y and adenosine, which may potentiate the effects of catecholamine on the cells. The balance between the transmitter and the co-transmitter may be important in determining the effect of the sympathetic system on the cell".

De Champlain said it is important to understand how sensitive the cells are to the neurotransmitters and how the cells will respond to them. The transmitters have several types of receptors, some with opposite reactivities.

"Just to give you an example, beta 2 receptors will cause vasodilation (dilation of the blood vessels). They are activated by norepinephrine. Alpha receptors will cause vasoconstriction. Yet they also are induced by norepinephrine. So the balance between those two types of receptors may be the clue to explain why the blood pressure increases in hypertensives. In fact what we find in both experimental hypertension in animals and in human hypertension is that there is a decrease in the beta receptor sensitivity, which is vasodilating, and an increase in the alpha functions, which are vasoconstrictive. So there is a real imbalance that occurs in the cell's perception of the sympathetic neurotransmitter in favour of an alpha dominance, which favours vasoconstriction. This is a quite recent finding. In the rat we can verify this hypothesis by measuring the cellular messenger activated by the alpha-1 receptor, and we are finding a marked increase in production of the second messenger linked to the alpha-1 receptors, whereas the production linked to the beta receptors is reduced.

"Now to verify the beta receptor response in humans we have started, in collaboration with Dr. Michel Bouvier, to study human cells. We are taking lymphocytes, which contain beta receptors, and we are measuring the beta receptors on those lymphocytes to see if they are reduced or less functional in hypertensive humans. If these receptors are impaired on lymphocytes that you take from the blood, then we can presume that they may also be impaired in the vessels.

"Using those approaches we may better understand the effects of some hypertensive drugs that have been used empirically over the years. People talk about drugs that contain so-called calcium channel blockers, which block the calcium channels by which calcium enters the cell. But what does the drug do to the sensitivity of the cell to the sympathetic nerves? Maybe the effect of calcium channel blockers is to reduce the sensitivity of the cell to sympathetic activation. That's one step forward towards the understanding of existing drugs, but also it may give clues to the development of new drugs that could be more specific to correct abnormalities in some basic mechanism that we could observe in the cell.

"With those methodologies using the rat model, we are learning how to modify the sensitivity of those various adrenergic receptors, and we're trying to find means to interfere with the imbalances that develop. We are lucky in these projects to be able to collaborate with one of my previous students, Dr.

Michel Bouvier, who is a specialist in adrenergic receptor modulation and is also an MRC scholar and grantee".

Another important area that the group has begun to study is the interaction of hormones with the sensitivity of receptors. One hormone that may be important is insulin.

"There has been an insulin resistance reported in hypertension which leads to increased circulating levels of insulin", said de Champlain. "Insulin is also a very powerful trophic factor (a factor that induces cell growth): it increases protein synthesis in the cell, thus producing hyperplasia (enlargement). So it is possible that in hypertension, insulin and norepinephrine work hand-in-hand to induce the major complication of hypertension which is cardiovascular hypertrophy (enlargement of the heart and thickening of the vessel walls)".

Although this effect of insulin has been known for years, no one until quite recently has studied the interaction of the two systems: de Champlain is doing that now.

In speaking of the body's control of blood pressure, de Champlain relates it to the thermostat in the heating system of a house. In hypertensive individuals, he says, "there is some evidence suggesting the thermostat is not

"...That's one step forward towards the understanding of existing drugs, but also it may give clues to the development of new drugs that could be more specific to correct abnormalities in some basic mechanism that we could observe in the cell..."

properly adjusted, so that it sets the blood pressure at a higher-than-usual level. It's just as though your house thermostat was fixed at 90 degrees in winter: even if you tried to decrease the heat in the room to 70, it wouldn't work".

De Champlain and Dr. Réginald Nadeau have been cooperating in research since 1968. Their interests are interrelated, because the sympathetic system is involved both in arrhythmias (Nadeau's chief interest) and in hypertension. Both were involved in establishing the Research Centre of Montreal's Sacré-Coeur Hospital, as described in Chapter 12.

De Champlain never set out to become a researcher. After graduating as an MD, he decided to take only one year of clinical research "just to see what it was like – I wanted to go into neurology".

He became interested in what he was doing and followed on with a PhD in investigative medicine. "After completing my PhD in clinical research, I wanted to understand basic mechanisms and so decided to go into basic science for three years of postdoctoral studies. I was lucky that I studied with a researcher by the name of Julius Axelrod who later, in 1970, won the Nobel Prize. Axelrod was a biochemist who discovered most of the mechanisms related to the biosynthesis, metabolism, uptake, storage and release of norepinephrine by sympathetic nerve endings. His stimulating influence determined the orientation of my research career".

De Champlain was surprised that he ended up in research, because he still very much enjoys clinical practice. But that turned out all right:

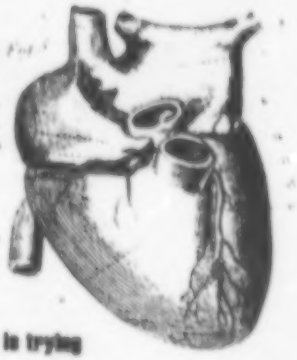
"When I do my clinical research, of course, I see my patients, and follow up on them. So I have the best of both worlds".

CHAPTER 9

"...All these new techniques started in the laboratories of physicists and physical chemists and were used there often for decades before an application could be found."

WHEN TWO HEARTS ARE BETTER THAN ONE

Absence may make the heart grow fonder, as the saying goes, but overload makes it grow larger. And while having "a big heart" in everyday parlance is a good thing, in cardiology it can be dangerous. It's called cardiac hypertrophy. Cardiac hypertrophy can result, for example, from hypertension: when arteries become narrow as a result of arteriosclerosis, blood pressure rises and heart muscle cells grow larger as the organ attempts to cope with the increased difficulty of pushing the blood through the circulatory system. Such a condition can lead to heart failure. But enlargement of the heart is not always bad: in the athlete the heart can grow bigger during intensive training yet still function normally – and when the intensive training stops it can return to normal size. Dr. Bertvaj Korecky, of the University of Ottawa's Department of Physiology, is trying to find out what exactly happens when the load on the heart is increased. What is the final outcome in the two types of hypertrophy: "Physiological or adaptive hypertrophy (athlete's heart), which is probably the good guy", as he puts it, "and pathological hypertrophy, which may be the bad guy"?



He's doing this with the aid of a laboratory "model" that consists of a rat with two hearts: the rat has a normal heart plus a cardiac "isograft" – a second heart taken from another rat and transplanted into the model's abdomen. The isograft is spliced onto the model's circulatory system by attaching the stumps of its aorta and pulmonary artery to the abdominal aorta and inferior vena cava of the recipient. Because it has its own internal pacemaker, the transplanted heart continues to beat, but at a slower rate than the model's own heart.

What this allows Korecky to do is to vary the pumping load on a functioning heart (the transplant), without affecting the rest of the circulatory system. In the technical terminology of a scientific paper, Korecky put it this way: "The transplant functions as a denervated 'non-working' Langendorff heart and does not appreciably contribute to the hemodynamics, while the recipient *in situ* heart supports the hemodynamics and serves as a control heart".

"...The old classical research at the organ or tissue level will still have problems to solve, but the underlying mechanisms probably mostly will have to be solved at the sub-cellular level by these techniques".

Korecky said the problem involved in trying to study the effect of load on the heart in an animal is that changing the load elicits all kinds of reflexes in the body: it's these, plus some sort of soluble growth factor, that are responsible ultimately for cardiac hypertrophy.

"What is important", said Korecky, "is to determine to what extent in each case the hypertrophy is due to direct load imposed on the heart – mechanically, for instance – and to what extent additional factors like cardiovascular reflexes and the release of circulating growth substances may be responsible".

In the case of hypertrophy caused by high blood pressure, some of the components of the heart usually do not grow proportionately, said Korecky.

"The extreme case would be that, for instance, the interstitial part – the part between the heart cells, which holds the organ together in the way the skeleton forms a framework for the muscles – and particularly the collagen structure, would increase more than the total mass. This can lead to a relatively stiffer heart during the relaxation phase of the heartbeat (diastole), so that when the ventricles have to be filled, they encounter increased resistance.

"This reaction of the heart becomes counter-productive. Ultimately these fibroblasts, which produce the interstitial fibre systems, can overgrow so much that they practically choke the cardiac muscles.

"In the case of hypertension, we sometimes reach a situation where even if we reduce the blood pressure, the heart remains large: these structures, which constitute the interstitium, regress only very slowly. We are trying to find out now which of the humeral factors might be responsible for this excessive growth of the interstitium. We believe from clinical observation and from

our experimental data that we have to define the enlarged hearts in a more meaningful way, in order to clarify which are the good adaptations and which may be an over-adaptation or a process leading directly to damage of the pumping function".

In the isotransplant model, the transplanted heart does not support the hemodynamic load and does not produce cardiac output, while the rat's normal heart does.

"We can now specifically overload the heart that is in the abdomen", said Korecky. "For instance we damage the aortic valve of the heart. The heart *in situ* is the control and beats at its regular rate and produces its normal cardiac output. So for the first time we have a model where we can selectively affect the heart and not affect the whole organism itself. Two hearts, one supporting the hemodynamic load, the other beating at a relatively low load, which can be increased. The load on the second heart is the blood flow running through it – say one-fifth or one-tenth of that of the other heart.

"In this transplanted heart I can either create valvular defects in the aorta and overload it", said Korecky, "or alternatively I can study the effect of humeral substances, let's say thyroxin, which I give to the animal. This will affect all kinds of tissues and also increase cardiac output and cardiovascular reflexes. However, the transplanted heart is denervated, so I can study the direct effect of thyroxin on the heart. I can see how some of the changes in the myosin molecule, which is the principal contractile protein, are affected directly by thyroxin, while others are affected indirectly through the cardiovascular reflexes".

At present this is the only way of doing this, other than in tissue culture, said Korecky. His laboratory model has the advantage that it uses a whole organ.

The isotransplant model has been used for some 20-25 years, Korecky says, but usually in immunological studies to test tolerance of an animal to a transplant. If the animal rejects a transplant, either the heart's pacemaker or the heart muscle becomes damaged and the heart stops beating.

"Our problem with the heart is that the heart cells, as with cells in the brain, are terminally differentiated: once they are committed to be heart cells they cannot undergo mitosis – they cannot proliferate", Korecky explained. "This has enormous advantages in that you rarely get any kind of tumorous growth in these organs: you rarely have a primary tumour of the heart or the brain. If you have one it probably originated somewhere else. This is how these vital organs are protected by Mother Nature.

"It is also of course a disadvantage if you damage those cells. If you kill them they just can't heal: they heal only by scar formation. This is why we occasionally do experiments to try to stimulate the growth of these cells: so that they could eventually replicate and replace those cells killed. But so far we have been singularly unsuccessful.

"They naturally can proliferate up to a point in the normal course of development. But by the time proliferation stops you have all the heart cells you're going to get for the rest of your life. We are trying to find out if in some way we can duplicate that process. Mother Nature locked those cells for obvious reasons, and even if somebody may some day succeed in unlocking them there'll be another problem: how to stop them. If heart or brain cells started to proliferate out of control, the organism would be finished – particularly the brain because within the skull there is no room for growth!"

Korecky says a genetic code must exist that starts, stops and locks in this process at a certain time in development. If that could be found and turned on and off, it would be particularly useful in replacement of damaged parts of the heart. But it would be extremely difficult.

"We've begun in a rather primitive way: we try, for instance, to induce extra proliferation about the time of birth and hope those cells that proliferate in excess may eventually come in useful. If, let's say, you normally have X number of cells and we could have 1.5 times X number of cells and still have a working heart, and if later on a certain portion of the cells were damaged, then this extra pool of cells might kick in".

Such an approach, says Korecky, will not be taken by organ physiologists like himself, but by molecular geneticists, and it will probably have to be worked out in tissue culture first.

Korecky obtained his medical degree at Charles University in Prague, Czechoslovakia, then took a PhD in human physiology at the Czechoslovak Academy of Sciences, and in 1966 settled in Canada.

About the new tool of molecular biology he says:

"It opens up completely new vistas and probably in many cases it will be absolutely essential to use this approach. The old classical research at the organ or tissue level will still have problems to solve, but the underlying mechanisms probably mostly will have to be solved at the sub-cellular level by these techniques.

"When it comes to application of its findings we will again need testing at the organ level and the level of the whole organism – and this will have to be done by someone other than the molecular biologist".

Korecky sees medical researchers like himself as highly dependent upon those in other, non-medical fields – especially physics – for the development of new laboratory techniques. Examples are the development of pressure and flow measurement techniques for use in experimental animals, and later in humans. He cites new imaging techniques that allow study not only of such things as pressure and flow but also metabolic mapping of the heart *in situ*, such as nuclear magnetic resonance spectrometry and positron emission spectrometry.

"Apart from molecular biology all these new techniques started in the laboratories of physicists and physical chemists and were used there often for decades before an application could be found. Physicists, engineers and enlightened clinicians made these applications.

"The classical example is echocardiography, which was done during the second world war by a young physician who worked in a sonar unit of the US Navy and later became a cardiologist. He always figured that if we could identify a submarine in the middle of the ocean, we should be able to look in a similar way at the heart and find out how that heart moves, what it looks like and so on. In developing echocardiography he began by borrowing a sonar from the navy and trying to look at the human body".

In echocardiography an impulse of ultrasound is directed at the part of the body to be investigated. Information about the position of the organ can be determined from the reflection of the ultrasound waves.

"This is now the premier technique of any tertiary health care centre", said Korecky. "In a simple form this technique is used, for example, by gynecologists to determine the location of the embryo in the female body. So something that was esoteric – developed to chase submarines – now chases fetuses and organs in the human body".

"...It has restored all the excitement and I think that's when you can progress the most. You've got to have fun in research. If you are not having fun it's hard to be creative..."

BIG-HEARTED SYRIAN HAMSTERS

About 40,000 people each year in Canada are diagnosed as having cardiomyopathy (disease of the heart muscle). This is a degenerative disease: it gets worse as time goes by. It generally leads to cardiac hypertrophy (enlargement of the heart), with its characteristics of breathlessness and swelling of the legs. Finally, as the heart muscles lose strength and their ability to pump blood, it culminates in congestive heart failure. About half those with congestive heart failure die within four years. The number of patients in Canada with this diagnosis has tripled since 1970. Cardiomyopathy can be caused by decreased circulation (ischemía), as a result of viral or other infections of the heart, or it can be what doctors call idiopathic (meaning they simply don't know the cause). One of the reasons doctors are seeing more and more people with these kinds of condition is that they have become successful at treating other forms of heart disease. Dr. Michael J. Sole, Director of the Division of Cardiology and the Centre for Cardiovascular Research at the Toronto Hospital and Professor of Medicine and Physiology at the University of Toronto, observes wryly: "As physicians we've been extremely

successful at converting acute disease to chronic disease, so patients with ischemic heart disease are becoming progressively an increasing proportion of the burden. We haven't cured them. We've created chronic heart disease where they would have dropped dead before".

But even the increased life-expectancy in late-stage congestive heart disease, though doubtless valuable to our patients, does not amount to all that much – perhaps two to four years, said Sole. New drugs and strategies have helped in making these patients more comfortable and giving them a much better quality of life. But it "has not really made any major inroads into mortality", Sole said.

It is for this reason that he and his associates are going right back to the beginning and investigating how heart muscle disease develops. As he and Dr. Choong-Chin Liew put it in a cardiology journal article:

"Trying to preserve cardiac function at the end stages of heart failure must ultimately prove to be a fruitless endeavour; it simply cannot be done over the long term. The solution to this clinical problem lies in defining and preventing, or arresting and reversing, the root causes of myocardial dysfunction".

In this quest they are being helped by an unlikely ally: a small animal known as the cardiomyopathic Syrian hamster. This rodent's value lies in the fact that it suffers from a form of heart disease very like that found in humans. And since the disease is transmitted genetically and arises spontaneously, and the animal lives less than two years, it constitutes an excellent laboratory model for the study of human heart failure.

Cardiomyopathic Syrian hamsters show little evidence of heart disease during their first two months of life (which correspond to adolescence in the human). But at about that time, patches of their heart muscle cells and chambers begin to die. These lesions begin to decrease at about three months of age, although they continue to form at a reduced rate as the heart responds by growing larger, and they are replaced by fibrous, calcified deposits. Death comes prematurely after 7-14 months, depending on the strain of hamster. Approximately one-half of the deaths are sudden and appear to result from cardiac arrhythmias (abnormal heart-beat rhythms) while the remainder die of congestive heart failure.

The parallels with human congestive heart disease are many, and Sole and his colleagues have been using the model for some 19 years. The hamster is not a Canadian discovery, but Sole says a disproportionate amount of work with this model has been done in Canada. Dr. Gaëtan Jasmin, a Montreal pathologist, was involved in developing it, and a major breeder is found in Canada.

One of the advantages of using this animal as a model is that surgical intervention is unnecessary: the researchers do not have to try surgically to reproduce in the animal the disease conditions they are studying, because they appear naturally. This avoids creating, through surgery, complications that have nothing to do with the disease process.

As with other disease models, the hamster/human match is not a perfect one. But, said Sole:

"Over 19 years the hamster has shown itself to be predictive of various abnormalities that we've subsequently found in humans. The concept of microvascular spasm – a spasm of micro blood vessels destroying muscle cells – first arose in the cardiomyopathic hamster, and has since been shown to be important in several forms of heart disease. Although there's no exact human correlate of the hamster disease, the hamster gives us a window into pathological processes".

Sole's laboratory is currently focusing on the molecular biology of heart muscle disease. He as a cardiologist and chemist and Dr. Liew, a molecular biologist, were the first to describe the genetic sequence of the human gene for the myosin heavy chains: myosin is a major protein involved in the structure and contraction of the heart muscle, and the heavy chains are key components of it.

"We are one of the few labs in the world currently looking at the nuclear proteins – proteins found in the heart cell nucleus – that are important for the regulation of cardiac gene expression", said Sole. "We've discovered some abnormalities in both human and hamster cardiomyopathy which appear to be identical. And we've identified one of those proteins, which appears to be important for the cardiac gene expression. We're now studying what role that protein plays".

One area that began as a side issue has resulted in an important development published in the July 1990 issue of the American Heart Association's journal, *Circulation*. Drs. Robert S. Ross and Kenneth R. Chien of the University of California-San Diego School of Medicine in La Jolla, California, wrote in a commentary accompanying the article by Drs. Ou Jin, Sole and Liew that the discovery "may lead to a new horizon in clinical cardiovascular research".

"We became interested in inflammatory heart disease, where it appeared that heart disease caused by a virus infection could be a precursor to cardiomyopathy", said Sole. "And we developed a diagnostic test for viral heart disease, using gene amplification by the polymerase chain reaction, which can be easily

adapted to the regular clinical laboratory at most hospitals. It turns out it's the first application of a polymerase chain reaction in heart disease of any kind".

Polymerase chain reaction (PCR) is a powerful new technology already used in criminal investigations to match blood or semen samples. Developed by Cetus Corporation of Emeryville, California, PCR makes it possible to find evidence of earlier viral infections of the heart by "amplifying" or multiplying genes or fragments of the DNA (deoxyribonucleic acids) that comprise them. Using PCR, a mirror image of the DNA is first made and then, in a chain reaction, perhaps a billion copies are produced. These can then be identified by means of biological probes.

"Using PCR, if there is even one viral particle present (in heart muscle), you should be able to detect it", said Sole.

"... I think it indicates how important mentors are in life. Many of us who come through school are basically still raw clay. It shows how important it is to try and recognize and identify people with potential..."

Sole's team screened small samples of heart muscle taken by means of a catheter from the heart muscle of 48 patients suspected to have myocarditis, an inflammation of the heart muscle, or dilated cardiomyopathy, an enlargement of the heart that sometimes follows this inflammation. In five of the 48, PCR showed enteroviruses had been present – a family of viruses believed to be common in development of myocarditis.

Sole believes viruses cause most cases of myocarditis. PCR will make it possible to identify these viruses in hospital laboratories. Sole's team is working with a major pharmaceutical firm on the development of a kit for the purpose. They hope PCR will reduce the number of biopsies necessary to detect myocarditis.

A puzzling feature of congestive heart failure is the fact that the body's responses to its development seem to be inappropriate and deleterious, for example increasing heart size and retaining fluid. Why should this be so?

"It turns out that these responses originated teleologically as responses to poor organ perfusion of blood or decreased cardiac output, in response to blood loss or fluid volume loss through dehydration", said Sole. "They were primitive responses of the body to try and maintain the circulation under those circumstances.

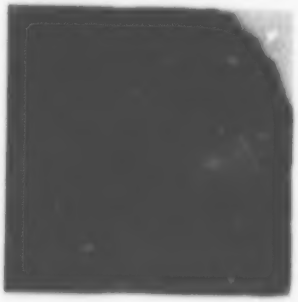
"But under those circumstances you have a normally functioning pump, or heart. What we have in heart failure is poor organ perfusion, poor circulation, because of an abnormality in the pump. Well, the body doesn't know why the circulation is depressed and begins to invoke responses that are designed to retain salt and water, build up volume, and so on.

"Congestive heart failure wasn't a survival issue for our ancestors or for more primitive animals. And what we've developed now in our society is a

failing pump. Fortunately, our understanding has let us intervene in these reflexes, which are deleterious, and have made life much easier for our patients. But they have not increased longevity".

As Korecky pointed out in the preceding chapter, once cardiac muscle has been damaged it has no ability to reproduce itself.

"The number of heart muscle cells you have once you're a few weeks old is all the heart muscle cells you're going to have for the rest of your life", said Sole. "So one direction in research is to try to understand why the heart is locked into this number of cells, and to find if there is a way of getting heart cells to multiply, because then the heart could begin to repair itself. And secondly, does the secret of why heart muscle cells turn themselves off explain why a cancer of the heart is almost unknown except in infants, where cells can still replicate? Maybe that would give us some clues as to why cells replicate in a totally uncontrolled manner, as in cancer. That whole area is very interesting".



Sole believes that when scientists get into any situation where they suddenly develop the ability to dissect a process at smaller levels, they go through a phase where it appears that they are only accumulating microbits of understanding. And only with an accumulation of those microbits can they develop a more general understanding. In Chapter One we saw how he applied this idea to particle physics, and by analogy to heart disease. Another example, he said, are the proto-oncogenes – sequences of DNA that, when altered, can cause the cells to become malignant.

"We began to accumulate more and more of these oncogenes, but also began to realize that they were part of a very important family of regulators of normal cell growth and development. And that's why their alterations either were lethal or resulted in malignant transformation. It's just that they were so fundamentally important that they couldn't withstand genetic change".

Sole began scientific life as a physical chemist and first published in that field. But "somehow or other I just didn't quite feel fulfilled. I don't know why, it must have been my personality. I went into medicine, and as I did so, for some strange reason – something that's continued to bother me – it began to snuff out my interest in science, although before that I was passionately interested in science. I was given keys to the lab when I was in high school, and I used to do experiments in rockets – that was in the Sputnik era.

"I soon began to see myself as going into the practice of internal medicine. I didn't see an academic life for myself at all. When I entered my residency, Doug Wigle, who is a well-known cardiologist, heard about my physical chemistry background and said 'anybody who's got a background in

research is not going into practice – you're going to become an academic cardiologist'. And I liked cardiology anyway. So he actually helped me write my first fellowship application and sent me on to the Cardiovascular Research Institute in San Francisco – and my research career started".

Sole came under the supervision of Dr. John Clements, the discoverer of the surfactant that keeps the lungs open, and Dr. Julius Comroe, a great science educator. He then got a position at Peter Bent Brigham Hospital at Harvard University and continued training there.

"Although I was doing research, I wasn't really that excited, until one morning, for some reason – it must have been related to a patient of mine – I woke up trying to figure out why, when the heart would seem to need its neuroregulation most, in heart failure, its neuroregulation became dysfunctional. I wondered why the organism would respond in that way.

"Once again I had a mentor, Dr. Ed Sonnenblick, who rather than confining me to his program, said 'Well, there's a real expert in neuroregulation over at MIT, Dick Wurtman, why don't you go see him'. So I went to him and proposed an experiment which worked out and, even though I was assigned to Harvard's system at Peter Bent Brigham, my supervisor there said 'Why don't you go over to MIT and work on these experiments'. So, even though he was paying me, I worked somewhere else and had the benefit of a Harvard-MIT education, which was wonderful and just opened my whole world".

Sole returned to Canada and devoted his initial 12-13 years of work to neurohormonal regulation of the heart.

"When I completed the studies that really had interested me and had in essence finished the story I wanted to tell, I was publishing papers but not having a lot of fun any more. I really wondered at that time – 1980-84 – if it wasn't time for me to hang up my skates. My philosophy was that if I wasn't having any fun, why should I do it. And at that point I had two choices: become more of a clinician or more of an administrator. Ironically I'm doing more administration now than I ever dreamed of".

At that time molecular biology was beginning to develop and intrigued Sole, so he switched careers from neurochemistry to neurobiology, which he calls "as different as apples and cows". He temporarily lost his long-term funding to short-term grants, and everybody thought he was crazy, except his wife. But in the end, Sole says:

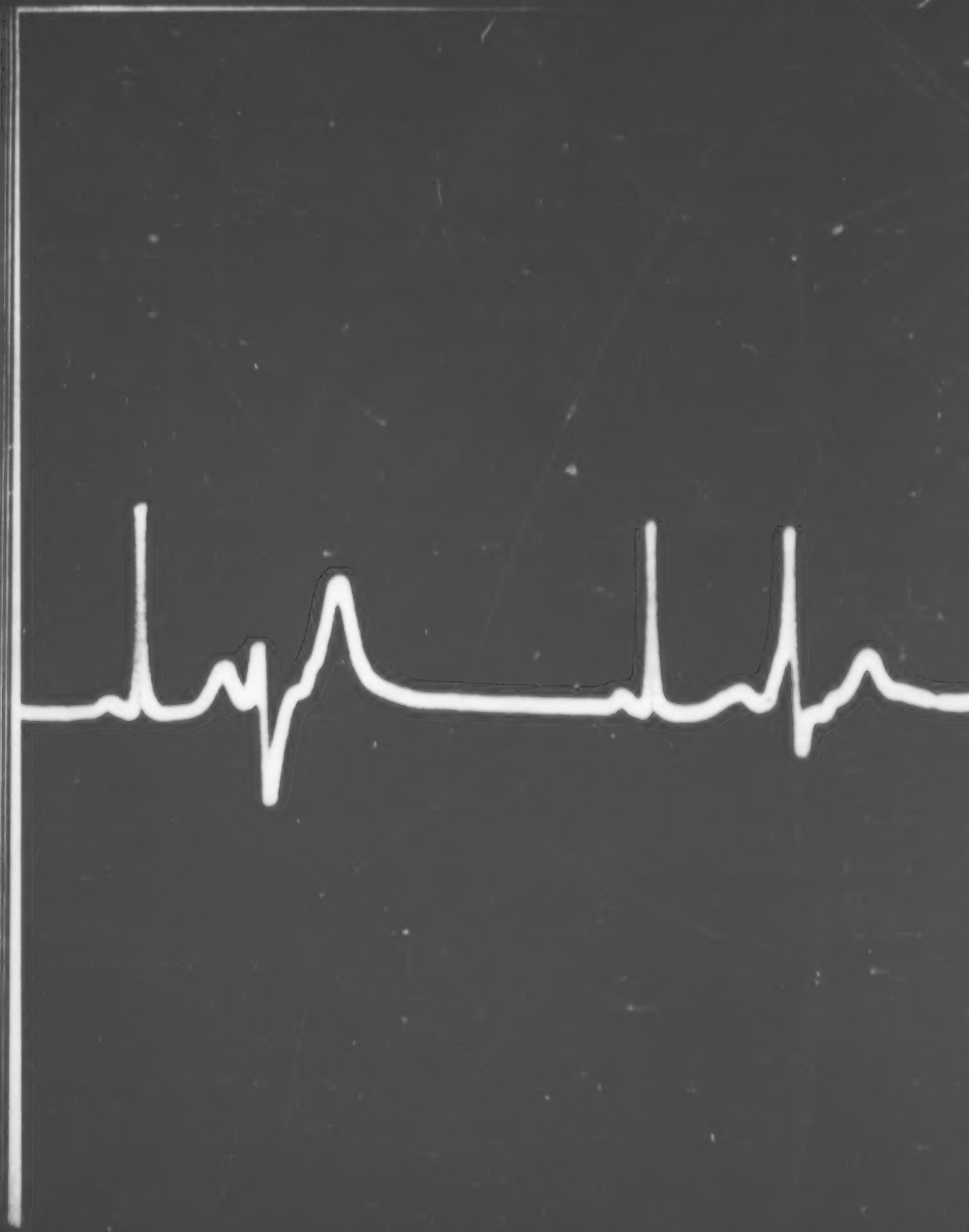
"Everything has worked out fine. It's restored the excitement and I think that's when you can progress the most. You've got to have fun in research. If you're not having fun it's hard to be creative".

His problem now is that because of his broad background in a variety of fields, he's caught in major administrative positions.

"I think one lesson I'm learning and struggling with is that I find you can teach and do research, you can do clinical work and do research, but it's very hard to do major administration and do research. Because administration competes for your creative time, and rather than go to bed with a research problem that you struggle with as you sleep, you wind up with an administrative issue. It's something that some of our best researchers probably face in their careers".

Are there lessons for others in his experience?

"I think it indicates how important mentors are in life. Many of us who come through school are basically still raw clay. It shows how important it is to try and recognize and identify people with potential. You can make whole career changes. People often don't know what they want when they are in medical school. They happen to like the last rotation they have, and think they have choices and understand what the choices are, but really without the broader perspective that an older mentor has, they may be unable to see what may be the most appropriate path for them".



THE ULTIMATE MYSTERY: SUDDEN DEATH

Sudden cardiac death is one of the commonest causes of mortality in Canada: it kills between 35,000 and 40,000 Canadians each year, making it a major public health problem. "Among the people who die from cardiac disease in Canada, about 42% of the deaths are heart and stroke together", says Dr. D. George Wyse. "Of that 42%, about three-quarters have ischemic heart disease (restriction of the blood supply to the heart). And in half of these, the mode of death is sudden cardiac death, meaning either no symptoms or very few, or symptoms followed shortly by death – a short enough time that they don't realize what the symptom is or seek medical attention". Most people tend to think that such deaths are caused by a 'heart attack', or in medical parlance acute myocardial infarction. A blockage interrupts the blood supply to the heart and the heart stops beating. The majority of sudden cardiac deaths, however, are caused by a sudden increase in heart-rate (tachycardia), so fast that the heart is unable to pump blood. Such a condition may lead to ventricular fibrillation, where the heart-beat becomes not only rapid but chaotic, so that the heart can no longer contract and pump in a co-ordinated way.

" Another area where we are involved is in measuring whether or not drugs are effective. Treating cardiac arrest is not like treating headaches..."

Many instances of ventricular fibrillation do indeed result from a myocardial infarction. But in 83% of those who survive the episode, no evidence of acute myocardial infarction can be found. Such patients – unlike those who have had a myocardial infarction without fibrillation – are at great risk of a recurrence from which they will die. In fact, 43% of them die suddenly, usually within a few minutes of the onset of fibrillation, during the two years following the first episode.

Great advances have been made in treating such patients in recent years. Mobile paramedic units in major population areas can speed to the spot and stop the fibrillation with the aid of an electronic device. And because of the high rate of recurrence among the victims, new and aggressive management techniques have been developed to prevent sudden death after the first incident. A team funded by MRC, composed of Dr. Stuart J. Connolly and Professors Michael Gent and Robin S. Roberts at McMaster University, has organized a group of investigators who will evaluate two principal preventive treatments. These are the surgical implantation of an automatic electronic defibrillator, and the use of a drug called amiodarone. Involving 400 patients and 28 co-investigators in 17 medical centres across the country, over five years, the Canadian Implantable Defibrillator Study will be an important step in learning how best to manage those prone to sudden cardiac death.

"What we're interested in right now is a group of patients at very high risk for sudden death", said Dr. Connolly. "They're high risk because they've had a sudden death already, but they got resuscitated, either by luck or by good planning. When it's by luck it's usually because it happened near a hospital or near an emergency room or something like that. When it's good planning it's because they lived in a city that had mobile paramedic units where people can race out and give them an electrical shock and save them in that way. Toronto has one, Hamilton has one, but many places in Canada do not.

"These people who survive their cardiac arrest are at very high risk of a recurrence. The problem for the last decade has been how to treat them, and a whole branch of cardiology has arisen around this, called cardiac electrophysiology. Cardiac electrophysiology has some other interests as well, related to less lethal heart rhythms, but primarily this is the bread and butter of the work of those in the field".

Cardiac electrophysiologists have had a number of different drugs to work with but have been disappointed in their ability to prevent recurrences of cardiac arrest. The drugs also have significant side effects. And while they seem to have worked to some extent, they have not been uniformly successful.

patients using them may still die suddenly, presumably of arrhythmia. Usually the drugs work through a direct effect on the electrical activity of the heart. The drug considered the most effective is amiodarone. However, it also has some of the worst side effects, including fibrosis of the lungs in about two percent of cases, and thyroid and liver problems.

"Because of a perceived failure of these drugs, an electrical device was developed that can be implanted in the body", said Connolly. "This device is connected to the heart by means of metal mesh patches sewn on its surface. It can also monitor the heartbeat, and when it detects a tachycardia or fibrillation – which it does by means of electronic sensing through what is really a little computer inside the body – it can then prepare a large charge that can be delivered by these patches to the surface of the heart. This can correct an abnormal heart rhythm. These devices were introduced about a decade ago and now are more widely used because follow-up with patients indicated that the device does seem to prevent deaths. The impression also is that it prevents death better than any of the drugs, even amiodarone.

"Consequently this device has become in some parts of the world – primarily the U.S.A., which has a bit of a love affair with hi-tech – the so-called treatment of choice for patients who've had a cardiac arrest, whether or not drugs are used. This led us to ask the question, 'Is this really the best way to treat these patients?'"

So popular has the automatic implantable defibrillator become in the U.S.A. that it is used six times as frequently per capita there as in Canada. In 1988, only about 50 to 60 new implants were done in this country. Cost is one reason: implantation requires major open-chest surgery, which is expensive enough by itself. But in addition the defibrillator costs about $20,000, and it must be replaced every 2-4 years at a similar cost. Such expense has made Canadian government health authorities reluctant to provide the necessary funding, particularly when solid data demonstrating its superiority over drug therapy do not exist.

The implantable defibrillator does not prevent ventricular tachycardia or fibrillation: the shock it applies to the heart stops such episodes if and when they occur. The patient can feel the shock, which is usually very painful. Other possible complications are infection, erosion of the electrical leads, and malfunction of the device: these are not common, but if they occur they can be serious. In addition, the mortality rate of the operation is 1.5%.

"...These people who survive their cardiac arrest are at very high risk of a recurrence. The problem for the last decade has been how to treat them, and a whole branch of cardiology has arisen around this..."

"...Drugs are not necessarily our best weapon for that particular problem. In fact, compared to some other things, drug treatment of sudden cardiac death is not particularly rewarding..."

There is a further danger, in the minds of the McMaster team: that the defibrillator "could become so widely accepted that it will not be possible to subject it to the rigorous testing that any new and expensive treatment requires".

This is not a trivial consideration. Said Wyse, who will be a collaborator in the Canadian study:

"Right now in the U.S.A. devices like the implanted pacemaker have the reputation of being a blazing success. It seems they have decided that's the way to go and drugs are no good. And many probably think a trial such as Connolly is organizing is probably not worth doing. I think the data are very soft and a trial is very appropriate. Medicine in general – and cardiology maybe more than other specialties – has had a history of enthusiasm for new therapies said to be wonderful, yet when they are investigated more carefully there often seems to be disappointment. These things seem eventually to settle down into proper usage. There are many good examples[5]: coronary artery bypass surgery in the 70s was thought to be the panacea – in the 1990s we know that it is an excellent treatment but needs to be applied appropriately[6]. Angioplasty (insertion of a balloon in blood vessels to remove a blockage or widen a narrowing) is a wonderful treatment, but exactly where it fits in we'll probably know only after a few years. I think devices for arrhythmias are going to be the same. If you look at the figures – 35,000 to 40,000 sudden cardiac deaths in Canada – that's a big number. If one-third were treated with this device it would be a costly effort".

Connolly concludes: "Clearly the way to answer the question is to compare this treatment with the best conventional therapy which is, in our opinion, amiodarone. Therefore we undertook to put together this randomized clinical trial, in which patients who meet fairly specific entry criteria would then be randomized (if they gave consent) to receive the defibrillator or not. The patients who did not receive the defibrillator would have to be treated with amiodarone, and the patients would all be followed up until the end of the study to compare the rates of sudden death in the two groups. The current plan is to enter patients at the rate of 100 per year for four years, and then follow all patients to the end of the fifth year. The study begins in October 1990".

The Canadian study will be the first of its kind, and is unusual in that it compares a surgical to a non-surgical therapy. It was originally Connolly's idea, and he is the principal organizer.

"Other people helped me think through my ideas, especially Dr. Jack Hirsh. From a statistical and data management point of view, Michael Gent and Robin Roberts, who run a group involved in the management of large clinical

trials, have worked closely with me in every aspect and I couldn't do it without them. In addition, the protocol has been reviewed by all the different investigators in the study, and we've had to accommodate different views, so it really is a joint effort".

The first internal defibrillators were implanted almost a decade ago. The current device is under intense development, with new models coming out frequently. The goal now is to avoid the necessity for major surgery: it's hoped the defibrillator could be installed with a patch under the skin and some wires threaded through veins into the heart.

While the defibrillator implant has "caught on like wildfire in the past four years because of the impression that it works", and while Connolly's group also believes that it works, they want to prove it in a way that leaves no doubt in anyone's mind. They are also naturally a little sceptical of all the enthusiasm that has been generated by the device, even though they too are already using it.

"I saw a patient today – an elderly guy who developed a bad heart muscle from a viral inflammation a couple of years ago, and who had a cardiac arrest a couple of weeks ago", said Connolly. "We're at the point where we'll either put him on amiodarone or we'll put in a defibrillator. And he says, 'Doc, what should I do?' And I say, 'Well, basically at this point in time it's flip a coin: I don't know which is better for you'.

"If you believe the Americans and all the hoopla, put in a defibrillator, but we've had good experience with amiodarone, and this is a guy who would be at some risk going into the operating room and having his chest opened. The reason I like this research is that I'd really like to know the answer to the question. This patient has no preference and says 'I'm totally in your hands', and I don't know the answer.

"It would be easier just to put him on amiodarone. He'd be out of hospital in a few days. We've had good experience with it, and it would be a lot easier for him. But you know there's this very strong suggestion in the literature, based on follow-ups, that we can reduce the risk of his dying suddenly in the next year by as much as 80 or 90% with the defibrillator.

"The trouble is", said Connolly, "that the estimates in the literature are not very good because the comparisons between those with the defibrillator and those on amiodarone are not randomly selected groups of patients. They may be very different groups, with very different pre-treatment risks of dying".

Connolly first took a bachelor's degree in philosophy at McGill University, then a master's at Fordham University in New York, before taking medicine at McGill. He started medical school without any plans to do

"...What we created was the possibility of coming into the hospital environment and getting clinical research into application, rather than building a separate institute."

research, but in his fourth year "there was an opportunity to have some very specialized stuff thrown at us, and I definitely got turned on by a couple of courses I took". One course was in pharmacokinetics, which led to his interest in research in anti-arrhythmic drugs and arrhythmia management in general. He took his cardiology training at Toronto and Stanford University in California. One of the attractions in joining McMaster was the opportunity to do clinical trials.

Dr. D. George Wyse's research group at Foothills Hospital in Calgary also focuses on sudden cardiac death as its major clinical problem.

"We have a number of projects in that area", he said, "some very basic, having to do with sodium and potassium channels in the cells and how they work, how drugs that prevent arrhythmias work on these channels, and how to look after patients with these problems. We are focusing mainly on the mechanisms of anti-arrhythmic drug actions and methods for detecting people likely to have sudden cardiac death. How do we go about finding such people and drugs likely to be effective for them?

"Drugs are not necessarily our best weapon for that particular problem. In fact, compared to some other things like high blood pressure and angina pains and acute myocardial infarction where drugs are the main thing, drug treatment of sudden cardiac death is not particularly rewarding".

Wyse's group has been trying for some time to use combinations of anti-arrhythmic agents in treatment of this problem.

"We think the mechanisms behind arrhythmias are complex and multifactorial, so if you use an agent that attacks only one thing you can't solve the problem. So we've tried to use agents that have several different desirable features together. That's something on which we've probably done more work than many others.

"Another area where we are involved is in measuring whether drugs are effective or not. Treating cardiac arrest isn't like treating headaches: you can't say, 'Take this pill, come back in a month and tell me if you've had any headaches', and if that doesn't work you try something else. Because if you tried that approach with sudden cardiac death the next episode would be the last one. So you have to have some way of evaluating the drugs before they go out".

Wyse is both an MD and a PhD, and does both laboratory and clinical research in cardiac electrophysiology. The University of Calgary's program encourages what it calls the "mosaic structure" for research: "We have traditional departments and a curriculum based on organ systems. Rather than grouping research according to which department you're in, research laboratories are

grouped according to which body system you do your research in. This means the cardiovascular research group has physiologists, pharmacologists, cardiologists, surgeons, biophysicists, and so on, all working together".

Arrhythmias are a particularly important malady in the province of Quebec, where they are responsible for a major proportion of the 10,000 sudden deaths a year – more than in any other province. One of Canada's senior cardiac researchers, Dr. Réginald Nadeau, leads a study at the Centre de recherche of Sacré-Coeur Hospital, Montreal, that is not only elucidating the underlying mechanisms of arrhythmias, but also enables the group to identify areas of the heart in which arrhythmias arise, pinpointing them for corrective surgery. It thus provides an excellent example of how laboratory concepts drawn from basic research can be applied to human health care in the operating room.

The study makes use of computers to map the electrical activity of the heart, and is a result of collaboration between Nadeau and Dr. Fernand Roberge, who established the Biomedical Engineering Institute at the University of Montreal and was responsible for developing the computer systems.

The surgical group includes Drs. Pierre Pagé, the cardiovascular surgeon, René Cardinal, an electrophysiologist, and Pierre Savard, an engineer. During surgery, Pagé opens the chest and applies a cryoablation probe to the area of abnormal electrical activity. The probe is a metal tool carrying extremely cold (minus 60 degrees C) nitrous oxide, which kills the tissue it touches. By applying it to the heart tissue remnants identified by the computer map, the surgeon permanently stops their aberrant conduction of electrical stimuli, thus preventing tachycardia from occurring.

"Now new techniques are coming along as well", said Nadeau. "You can give an electric shock to destroy this little piece of tissue. It can't be done in all cases – it depends on the location. Once you have the site identified you put electrodes just on the inside of the heart and you give a shock like you do with a defibrillator, only the shock is given within the heart and the electrical energy is lower. Techniques using microwaves are also being tested".

Nadeau has always worked with a team of people in research, saying, "I'd rather consider myself as a coach than anything else". He became interested in research as a summer student after his second year in medicine at the University of Montreal.

"I had a wonderful professor of physiology whose name was Eugène Robillard. He kindled my interest in research and in electrophysiology. When he gave lectures on the electrocardiograph it intrigued me to know how the

"...With all these new techniques, it's a whole new ball game in the last five years. Things are going so quickly in cardiovascular physiology that it's hard to keep up..."

inventor imagined this system: what was the process that led to it? I always remained interested in that type of work".

After he finished medical school in 1957, Nadeau did research in physiology and then decided to get special training in clinical cardiology.

"I always was with people who taught me that part of being a physician was to do basic research and I trained with people who did half and half. It wasn't common then, and when I came back to Montreal in 1964 the first thing I was told by the dean and the people at the hospital was 'you must choose: you can't do both'. I said, 'I was trained to do both'. But I didn't find it very easy. Now the research centre here (at Sacré-Coeur) allows people to do both".

Nadeau spent his first years in the Department of Physiology at the University of Montreal under Professor Jean-Pierre Cordeau, who had begun as an engineer and served in the Second World War but later trained in medicine and then in neurophysiology.

"He wanted to get a good base of research going at the university. I owe a lot to him because he permitted me, coming back as a cardiologist, to go into the Department of Physiology and set up a little lab – he helped me to get going. And that's where I met both Fernand Roberge and Jacques de Champlain, with whom I've worked now for 27 years".

When Sacré-Coeur Hospital became affiliated with the University of Montreal, the three used the opportunity in 1972 to create a centre where research could be quickly applied clinically.

"We've worked very closely with people on the campus", said Nadeau. "What we created was the possibility of coming into the hospital environment and getting clinical research into application, rather than building a separate institute. It doesn't have the rigidity of an institute. The clinician and the basic scientist accept each other. But you have to work on that: it doesn't come spontaneously. You have to create the climate with the nurses and the hospital administration and everybody".

At the University of Sherbrooke, Dr. Jean L. Rouleau, the head of cardiology, who formerly worked with Nadeau's group, takes yet another approach to sudden death:

"We're interested in the evaluation, the risk profile, of patients after myocardial infarction. There are three types of major complications that can cause death after an acute myocardial infarction. One is congestive heart failure: the development of this is dependent partly on the size of the infarction, and also the response of the scar to the infarction – whether it stabilizes easily or dilates and whether you get a big, baggy ventricle or maintain a reasonably

good size of ventricle. There's been a fair amount of work done on this in the past but we are doing some animal work on it – basic science – and some human work. The second major complication would be a new infarction. The third way is by sudden death: arrhythmias.

"The three are interrelated: if you get a new ischemic event, you get arrhythmias. But patients who develop congestive heart failure and dilatation of their ventricle are at much higher risk of developing arrhythmias, so what we're doing is looking at what happens to the scar post-infarction, and how that may affect the risk of sudden death and arrhythmias. That's a basic science study in rats. We are also trying various drug interventions to attempt to modify this risk, or the susceptibility of the heart to arrhythmias. In humans, we also focus on the scar and what happens to it, and how that is related to arrhythmias and the susceptibilities of patients to arrhythmias and sudden death. And whether we can modify that with a drug".

Rouleau looks at the scar with echocardiography, which as Korecky related in Chapter 9 is an adaptation of techniques the navy uses to detect submarines. In a large population, he observes whether the response of the body to dilatation of the heart – which involves hormones – is also related to sudden development of a big scar, or to arrhythmias or sudden death.

"We're also doing a pharmacological intervention with the angiotensin-converting enzyme inhibitor captopril. It's been shown in animal studies and some pilot human studies to prevent dilatation of the heart. We're looking at the dilatation and what makes it occur, and what kinds of effect that may have on arrhythmias and survival. And the relationship that hormones may have to all this".

The project has been going on for three years and has two more to run. It involves by far the biggest base of patients ever accumulated anywhere on this problem (522 people from across the country). Directed by the University of Sherbrooke as an MRC university-industry program, it also has the participation of three centres in the U.S.: Peter Bent Brigham Hospital at Harvard University, Mount Sinai Hospital in New York City, and the University of Maryland hospital in Baltimore.

"Let's say you get an infarction – a big infarction. One patient has a hormone response and one doesn't. In some patients the ventricles dilate, in others, they don't. So we're trying to see what role these hormones may have in the dilatation. And then once dilatation occurs, some develop arrhythmias and die suddenly, and others don't. And we want to see whether the hormones play a role in that or whether dilatation plays a role in that. Some patients with

dilatation also develop heart failure and we don't know if the hormones are related to that or not. We believe so, but it's never been shown".

Rouleau says the data being obtained will not just increase understanding of the disease process, but also affect the approach to treatment. Angiotensin-converting enzyme inhibitors have been shown to be effective with animals, so his study is trying to see if other drugs can be too – or if they are deleterious.

The drugs are not experimental ones, but compounds used in practice, yet whose mechanisms of action are not fully understood. It's hoped the study will elucidate these mechanisms, after which the drugs – sometimes in combination – could be tried in specific situations in patients.

"There are a lot of things we do that we don't really understand", said Rouleau. "Beta blockers prolong survival, especially for people with big infarctions, but we don't know why. It's difficult to get a rational combination of medications if you don't really know how a drug works".

Another University of Sherbrooke study involves risk-factor profiling in eight university centres of all patients who have had a heart attack. Rouleau says some risk-factor profiling was done in the past, but the introduction of thrombolysis – the use of drugs to break up blood clots after a coronary blockage – in the early 80s, and its widespread use beginning five years later, has complicated the picture.

"Is what was useful before still useful today?" asks Rouleau. "I don't know. Thrombolysis may change things: it decreases the mortality after an acute infarction by 30-50%, so obviously there are a lot of those people who were going to die who are still alive. Now how does that affect what we used to do? We are evaluating the effects of all these new techniques and trying to see what are the important ways to identify patients at high risk: are they the same as before?"

Rouleau is a Franco-Ontarian who took his MD at the University of Ottawa and specialized in internal medicine. He took cardiology at McGill and learned about cardiovascular research at the Cardiovascular Research Intitute at the University of California (San Francisco). He began to do basic research at Montreal General Hospital and Sacré-Coeur Hospital in Montreal, then went to Sherbrooke.

He is unusual among the researchers interviewed for this book in that he had no special interest in science as a youngster. His motive for taking medicine was to help people; his motive for entering research was to find ways to do this better. Curiosity was part of it, but it was mainly a humanitarian

impulse. He was frustrated because most of what doctors did was to treat patients by recipes, without really understanding why the drugs they prescribed were expected to be useful. He enjoys the research process, but his real satisfaction comes from treating sick patients successfully.

[5] For examples of medical treatments that have received the support of mainstream medicine in their time, yet later were shown to be worthless or harmful, see *Modern Medical Mistakes*, by Edward C. Lambert, M.D., Bloomington and London, Indiana University Press, 1978; and *Great and Desperate Cures*, The Rise and Decline of Psychosurgery and Other Radical Treatments for Mental Illness, by Elliot S. Valenstein, New York, Basic Books, Inc. 1986.

[6] Valenstein notes: "In total, about 1,000,000 patients have had a coronary bypass operation, and, in an amazingly short period, it became the most frequently performed elective surgery in the United States...the total cost of these operations was about $1 billion in 1977 and over $1.5 billion by 1985. Together with the cost of all the supportive industry producing supplies and equipment, the money involved annually has been estimated to be $5 billion". See also Chapter 13.

As the preceding chapters show, medical research is producing large numbers of treatment options for people with heart disease. Sometimes several options are available for any given individual. For some patients, a choice may have to be made between a medical treatment and a surgical one. How can doctors – and their patients – choose between them?

CLINICAL TRIALS: THE PERFECT TOOL?

Perhaps the most important tool available to medicine in assessing the value of particular treatments today is the randomized clinical trial. "Nothing is perfect", said Dr. Martial G. Bourassa, who has acted as principal investigator in major trials for the Institut de Cardiologie de Montréal (ICM), "but with a well-planned and well-performed clinical trial you eliminate all bias and everybody will believe the results". Both Bourassa and the ICM have become well-known in international medical circles for their participation in clinical trials. One of the most important was the Coronary Artery Surgery Study (CASS), which was undertaken between 1975 and 1979 in 11 centres in Canada and the United States. The ICM was the only one involved in Canada and Bourassa was the principal investigator for the institute.

"...Nothing is perfect, but with a well-planned and well-performed clinical trial you eliminate all bias and everybody will believe the results..."

In CASS, 780 patients with defined categories of heart disease were randomly assigned to medical or surgical treatment in a trial organized by the U.S. National Heart, Lung and Blood Institute. The medical treatment was the best available at that time, and included drugs such as beta blockers, nitroglycerin and nitrates. The surgical treatment consisted of coronary bypass grafts. The primary aim was to determine what effect the different treatments would have on the survival rate of patients over a period of five years, and the conclusion was that surgical treatment did not improve the survival rate, except in well-defined subsets with left ventricular dysfunction or left main coronary disease. It did, however, improve quality of life through reducing chest pain, improving the patients' ability to function and reducing the need for drug therapy, compared with those receiving medical treatment.

This was one of the first large clinical trials for coronary bypass surgery, and as noted in the previous chapter, the procedure has become enormously popular, despite its great expense. Yet CASS showed that the patients who underwent medical treatment rather than surgical treatment survived just as long overall, and at much less expense. As the CASS study investigators put it in their official publication: "The decision to proceed with bypass surgery in these patients can be reduced to a question of whether the current level of symptoms is acceptable to the patient".

Another major trial on bypass surgery was carried out exclusively in U.S. Veterans' Administration hospitals, and a third in Europe. They are difficult to compare directly, but overall they showed much the same results: that patients with heart disease that involves a number of blood vessels but whose ventricles function well, usually gain nothing in survival time over five years with bypass surgery. However, surgery can improve five-year survival with those who have left ventricular dysfunction, narrowing (stenosis) of the left main coronary artery, or severe ischemia (blood supply deficiency) on exercise.

"There are many areas where clinical trials are important, and in fact are perhaps the only way to establish the value of treatments", said Bourassa. "Coronary angioplasty, which has become an important intervention, is another. We're participating in one now with the U.S. National Institutes of Health (NIH), comparing bypass surgery and coronary angioplasty. Again it is with patients with multi-vessel disease, to see whether one should start treatment with angioplasty in most of these patients and then resort to surgery if you have to, or if the results are better if you do surgery as a first procedure.

"The primary objective again is comparison of five-year mortality rates, but we're looking also at socio-economic costs and quality of life. We started in

1988 and will finish enrolling patients in 1991, and the study will be published in 1994. Initially we were the only centre in Canada in this study but The Toronto Hospital is now working with us. The study will involve 2,400 patients in 13 centres. So far about 200 Canadian patients have been enrolled, and another 75 will be added".

The study is called the Bypass Angioplasty Revascularization Investigation (BARI). Bourassa said at least three other clinical trials comparing angioplasty and bypass surgery are going on now, one other in North America and two in Europe.

Coronary angioplasty was first performed in 1977, but it really became more widely used only in the 1980s. Clinical trials did not begin until 1987. In the first three years or so medical personnel gained experience in the technique and technological changes occurred, and it was not until the mid-1980s that it was felt that the success rate was high enough to be acceptable, compared to bypass surgery, and the risk low enough. The success rate has climbed from about 70% in the early 1980s to 90-95% today, and even now new devices and catheters are still being developed.

"...I'm not sure we're doing enough clinical trials in Canada. There's been a strong emphasis at the NIH for the last 15-20 years, but the interest in Canada has been quite recent..."

The BARI trial uses balloon angioplasty, in which a small balloon is introduced into the blood vessel to reduce the obstruction by dislodging the cholesterol plaques. Other methods used in angioplasty are nonsurgical removal of the obstructed area (arteriectomy), the use of a laser to remove the obstruction, and endoluminal stents (placement of a support – usually metallic – inside the lumen of the artery to keep it open). These devices are put in place with a catheter. All of these procedures will have to be assessed in clinical trials eventually.

Drug evaluation is another area in which clinical trials are important. Many are currently going on with thrombolytic drugs, which are used to get rid of blood clots in patients who have suffered a heart attack; with anticoagulant and antiplatelet drugs used to prevent thrombosis; and drugs used for treatment of congestive heart failure, hypercholesterolemia and hypertension. Some of these are sponsored by pharmaceutical firms, and many by the MRC and NIH. The ICM is involved also in some of these.

Drug companies are primarily interested in testing new products for safety, so they can get them on the market, said Bourassa. This means short-term tests of 3-6 weeks. Few are really interested in getting involved in large clinical trials. However, one example in which a pharmaceutical firm is involved is in a trial of enalapril, an angiotensin-converting enzyme inhibitor manufactured by Merck, Sharpe and Dohme. Merck donated a large sum of money

for the trial to the NIH, with no strings attached, and NIH added some of its own money. The ICM is also involved in this study.

Still another project in which ICM is involved arose from its finding that atherosclerosis can develop in grafts of transplanted saphenous vein after bypass surgery. The trial – which is entirely NIH-sponsored – is designed to see if lovastatin, a lipid-lowering drug, can reduce the incidence of such atherosclerosis.

An important factor in clinical trials is the number of patients involved.

"You have to have enough patients", said Bourassa. "You have to have a sample size that is large enough to answer the question posed and to avoid errors. The size depends on what you are looking at. If you are looking at mortality you have to have a large enough sample to calculate current mortality in each of the two treatments involved. Some studies use 30,000-40,000 patients, for example those involving thrombolysis, where mortality is low".

Such studies rely heavily on computers and have developed mainly within the past 10-15 years. All data are collected and distributed through computer terminals: even mail among the participants is electronically sent and received.

"I'm not sure we're doing enough clinical trials in Canada", said Bourassa, who was a member of MRC's Clinical Trials Committee from 1984-1989. "There's been a strong emphasis at the NIH for the last 15-20 years, but the interest in Canada has been quite recent, during the last five years or so since the MRC has had its committee. This gave a big impetus to the whole concept. Having said that, there are few centres in Canada that are well-equipped to do them. They take important resources: biostatisticians, computers, a good relationship between doctors and biostatisticians, and a lot of money. Studies like CASS cost $35-$40 million. In Canada we're making do with much less".

Bourassa had no formal training in statistics: he trained himself through reading and talking to biostatisticians. He had taken his medical training at Université Laval, then went to Buffalo General Hospital for training in internal medicine and to the City of Hope Medical Center, Los Angeles, California, for cardiology. He took further training in cardiology in 1964 at the Montreal Heart Institute when it was part of Maisonneuve Hospital.

"I was interested primarily in hemodynamics when I graduated in medicine in the early 60s", said Bourassa. "I did a lot of cardiac catheterization and did the first coronary angiograms here in 1964-5". (Coronary angiograms involve the injection into an artery of a contrast agent which shows up on an x-ray, giving the cardiologist a two-dimensional view of the artery).

In 1976, he decided because of a health problem to abandon his former deadline-pressured existence with a big medical practice, research duties and work in catheterization, and to turn his attention to clinical trials. It meant a more ordered life without excessive stress – but with a great deal of challenge.

"One of the disadvantages is that it takes a lot of time", Bourassa said. "These studies often take 8-10 years". On the other hand, the results are usually so plentiful that they lead to many important publications. "The CASS trial, for example, resulted in about 100 papers. And you have the help of the best biostatisticians in the world".

Bourassa showed no particular interest in science as a youngster, but he was a great reader.

"I would read three or four books a week", he said with a smile.

From 1984-88, Bourassa was Chairman of the Joint Task Force on Coronary Angioplasty of the World Health Organization and the International Society and Federation of Cardiology. He also was the recipient in 1986 of the Grand prix de la Francophonie of the Académie Française in Paris.

EPILOGUE

As the conversations reported in this book show, Canada's medical researchers deal daily with matters that have life-and-death implications. What they discover about cardiovascular disease affects, in a very direct way, the quality and length of life of thousands of their fellow citizens. Both public and private funds have helped make possible their advances. The Medical Research Council of Canada and the Heart and Stroke Foundations provide the major share: in 1989 - 90, these two organizations set aside more than $42 million for cardiovascular disease research. Yet according to some of those who carry out this work, the sums provided are insufficient for them to remain at the cutting edge of knowledge in this area. There is no doubt that the federal funds available for biomedical research fall short of the demand. This year, although the MRC will spend some $220 million on all areas of research, including the heart, the greater part is already committed to ongoing projects, leaving limited resources for new ones. The result was that only 18 percent of the total of new requests for medical research grants could be approved, one of the smallest proportions ever.

"The support of science is a lot like parenting: it is not enough just to give money, you have to show that you care. And it's showing that you care that makes the big difference..."

This means that much valuable research will go unperformed.

International surveys have shown that Canada's position in research funding generally compares unfavorably with that of other developed countries. And we have one of the lowest proportions of scientists in the world: 4.4 per 1,000 workers compared with 8.4 in Japan and 6.6 in the U.S.A.

According to Dr. Yves Morin, Canada suffers from not having an indigenous pharmaceutical manufacturing industry to carry out expensive research. "We're the only country of our size without this", he says. He thinks the reason is that we have been shortsighted in our treatment of patent protection. There is a limit to what governments can fund in research, and a new drug may cost $200 million to develop. Yet he says Canada has penalized drug companies through its patent laws, despite the fact that they are currently the only expanding source of research money in the country. Morin does not fear control of research funds by industry as some researchers do, because so much of it is a search for basic knowledge and thus unlikely to be subject to direction for narrow commercial reasons.

Nevertheless, some researchers feel, as does Jacques de Champlain, that "if one wants to keep total liberty with respect to the type of topic you want to research, the MRC type of support is the best we can get. We can always work with drug companies on specific drugs."

The inadequacy of funding is not the only problem in cardiovascular research, however. The shortage of graduates wishing to go into research was a major complaint among those interviewed. Some could readily understand why this is so. Andrew Rankin noted that if medical graduates enter medical practice instead of research, they stand to earn perhaps three times as much – and they won't have to write a grant application every year, which is time-consuming and, in his words, "tremendously stressful".

However, Rankin, who comes from the United Kingdom, says the situation in Canada isn't bad by comparison with that country: "Aside from the fact that I prefer Canada to work in, there is no chance of getting a job in U.K. as a university professor. Whereas at least here I have the option of two or three universities".

Peter Olley referred to the results of a survey of medical students at his university: not one out of 120 replying wanted to pursue a research career. This is a pattern he sees every year, despite the fact that he is in a school with a strong research base. One difficulty he pointed to was that "we keep lengthening the training requirements... you're in your mid-thirties before you're trained".

John Ledsome thinks part of the difficulty in persuading people to go into medical research is that they don't know what it is about, because they have never been exposed to it. To remedy this, he says: "You can encourage people to do research before they go into medicine, or you can make research training more attractive or more necessary for an academic career in medicine." Another option would be to make the medical curriculum more flexible by providing an opportunity for those who think they might like to try research, for example in a program combining medicine with a PhD degree.

Ledsome said the MRC has done two surveys in the past five or six years, one on training of health professional researchers and the other on biomedical scientists with PhDs. Both studies were designed to elucidate the factors that influence interest and success in research. In both cases, the principal factor was found to be the experience in research gained during the students' summer employment as undergraduates.

"It makes sense: it gives them an exposure to research", said Ledsome. "Then they know what it's about and they can decide whether it's for them or not".

Michael Sole maintains that lack of opportunity is a major factor in reducing the number of young people interested in research careers in Canada.

"Historically, the department of medicine at University of Toronto has had only one or two MRC Fellowships for research trainees. Why should a great university like this have so few? It turned out that the opportunities for MRC Fellowships were so few that it just wasn't worth the effort to apply. Medical students would look elsewhere or fall by the wayside. ICI Pharma, the pharmaceutical company, set up six new fellowships in the department of medicine. The first year the program was slow getting off the ground and they didn't really get the publicity out, and so we wound up with one MRC fellowship and three ICI fellowships. But the next year year we had a doubling of applicants: everybody had heard about it and knew there was a good chance of getting six, maybe eight, fellowships and thus the odds were now worthwhile. We now had 26 applicants – far more than we had ever had – and the result was that we were awarded eight MRC plus six ICI: 14 fellowships. In other words a 700% increase.

"What we showed was that it was lack of opportunity that was killing the interest of our young in research careers, and that given the opportunity, there are bright people who meet MRC standards and who are excited about entering a research career. Some will still go through their research fellowships and go out into practice, but at least their exposure to research could improve

their ability to assess developments critically as they occur in medicine. I'm sure that, just as I changed my career when given the opportunity, some others will also do so".

Those who choose research as a career want passionately to do it, contends Sole. And for him, a passion for science is essential in making a country great – "a passion for excellence, to be first or leaders in some areas, to take a risk, even to waste money at times in the hope that something will come of it because that's what happens in creative research".

Sole spoke for many when he said: "We in Canada have a focus on the arts, but somehow or other we never celebrate science". A major purpose of this book has been to celebrate the scientific achievements of a small selection of Canadian medical researchers, in the hope that it may encourage others to emulate them in their passion for science.